(June 15, 2006)

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OVERALL SUMMARY FOR N-METHYL FORMAMIDE

Physical and Chemical Characteristics

N-Methylformamide (also known as monomethylformamide or MMF) is a clear, colorless liquid with a slight amine odor. MMF has a water solubility value of $1.0x10^6$ mg/L, has a melting point of –3.8°C, and boils at 199.5°C. MMF has a vapor pressure of 0.253 mm Hg @ 25°C, density of 0.9961 g/cm^3 @ 25°C, a flash point of 119°C, and flammability limits of 1.8-19.7%. **Data for physical and chemical characteristics are complete and no further testing is recommended.**

Table 1: Physical and Chemical Characteristics for MMF

Melting Point	-3.8°C
Boiling Point	199.5°C
Density	0.9961 g/cm^3 @ 25°C
Vapor Pressure	0.253 mm Hg @ 25°C
Log Kow	-0.97; -1.14
Water Solubility	$1.0x10^6$ mg/L
Flash Point	119°C
Flammability Limits	1.8-19.7%

Environmental Fate

If released to air, a vapor pressure of 0.25 mm Hg @ 25°C indicates MMF will exist solely as a vapor in the ambient atmosphere. Vapor-phase MMF will be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 57 hours. If released to soil, MMF is expected to have very high mobility based upon an estimated Koc of 0.0439. Volatilization from moist soil surfaces is not expected to be an important fate process based upon an estimated Henry's Law constant of $2.0x10^{-8}$ atm-m^3/mole. MMF has been shown to biodgrade using microorganisms obtained through soil enrichment. If released into water, MMF is not expected to adsorb to suspended solids and sediment based upon the estimated Koc. MMF, present at 400 mg/L, reached 4%, 98%, and 100% of its theoretical BOD in 3 hours, 3 days, and 7 days, respectively, using an industrial activated sludge inoculum and the Zahn-Wellens test; therefore, MMF is inherently biodegradable and expected to biodegrade in the aquatic environment. A ready biodegradation test following OECD guideline 301B indicated that MMF is readily biodegradable. Hydrolysis is not expected since amides hydrolyze very slowly under usual environmental conditions. Volatilization from water surfaces is not expected to be an important fate process based upon this compound's estimated Henry's Law constant. An estimated BCF of 3 suggests the potential for bioconcentration in aquatic organisms is low (HSDB, 2003). Consistent with behavior described above and assuming equal emissions to air, water, and soil, any residual MMF is expected to be distributed primarily to water (39.7%) and soil (59.8%) based on the Mackay Level III fugacity model. **No further environmental fate testing is recommended.**

Table 2: Environmental Fate for MMF

Bioaccumulation*	Low potential for bioaccumulation BCF = 3
Biodegradation	Readily biodegradable
Fugacity*	Air: 0.43% Water: 39.7% Soil: 59.8% Sediment: 0.073% Based on standard emission scenario: 1000 kg/h for air, water and soil
* Modeled data.	

Ecotoxicology

Existing aquatic toxicity test data can be found in Table 3. Modeling of physical/chemical parameters (i.e., Kow) and aquatic toxicity was conducted to help provide insight into the behavior in the environment and the aquatic toxicity of MMF. Syracuse Research Corporation models for estimating physical/chemical properties were used to estimate $\log_{10}$ Kow (Meylan and Howard, 1995) for subsequent use in the ECOSAR program (Table 1). ECOSAR (Meylan and Howard, 1999) was used to estimate the aquatic toxicity of MMF to green algae, daphnids (planktonic freshwater crustaceans), and fish. ECOSAR predictions are based on actual toxicity test data for classes of compounds with similar modes of action. The existing aquatic toxicity test data and ECOSAR predictions indicate that MMF is likely to be of low concern for acute toxicity to algae, invertebrates, or fish at environmentally relevant concentrations. **No further ecotoxicity testing is recommended.**

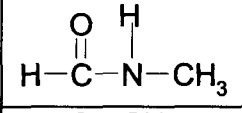
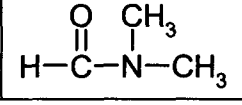
Table 3: Ecotoxicology

	MMF Test Data	**MMF Modeled Data**
Log Kow	No Data	-1.14
Toxicity to Fish (96-hour)	LC_{50} (fathead minnow) > 10,000 mg/L (N) LC_{50} (fathead minnow) > 5,000 mg/L (N)	LC_{50} = 39,170 mg/L (E)
Toxicity to Invertebrates (48-hour)	EC_{50} (*Daphnia*) > 500 mg/L (N)	EC_{50} = 33,787 mg/L (E)
Toxicity to Algae (EC_{50} value for growth inhibition)	72-hr $EC_{10\text{-}90}$ > 8000 mg/L (N)	96-hr EC_{50} = 17,630 mg/L (E)
E = estimated value, N = value based on nominal concentrations.		

Mammalian Toxicology

While reliable MMF data are available to address most of the mammalian toxicology endpoints, certain data are lacking. The available MMF repeated dose study was 2 weeks in duration, rather than the recommended 28-day study. While numerous developmental studies were available, limited data were available regarding reproductive toxicity. With regards to genetic toxicity, an Ames test was available for MMF, but no data on clastogenicity were available. In order to strengthen the mammalian toxicologic database for MMF, supporting data for DMF (a structural analog) are provided.

Table 4: Structural Analogs

Chemical	CAS Number	Structure
N-Methylformamide (MMF)	123-39-7	
Dimethylformamide (DMF)	68-12-2	

DMF is closely structurally related to MMF (see Table 4 above), in that both contain an N-substituted formamide moiety. The substances differ only in the degree of substitution on the nitrogen atom; MMF contains one methyl group and DMF contains two. Review of the toxicologic databases for MMF and DMF indicates that the two substances have generally similar toxicity profiles. For those areas where DMF data is being used as a structural analog to provide supporting data for MMF, detailed robust summaries are provided in this document. In addition, physiochemical properties of DMF are generally similar to MMF and are provided in the detailed robust summary format.

The pathways for biotransformation of DMF and MMF have been extensively investigated. Qualitatively, the pathways of metabolism for DMF and MMF are quite similar. The major pathway for primary metabolism of DMF is the P450-mediated oxidation to form *N*-(hydroxymethyl)- *N*-methylformamide (HMMF). An alternative pathway for biotransformation of DMF is formal demethylation to yield MMF. MMF is further metabolized by hydroxylation of the remaining methyl group to form *N*-(hydroxymethyl) formamide, or by oxidation of the formyl carbon, leading to formation of a reactive carbamoylating intermediate. The reactive intermediate can react with cellular glutathione (GSH) to yield SMG, which is eventually excreted in the urine as the corresponding mercapturic acid. A more detailed discussion of the metabolism of DMF and MMF is presented at the end of the toxicity section.

Mammalian Acute Toxicity

MMF has slight acute oral toxicity with an LD_{50} in rats of 4000 – 7077 mg/kg and an LD_{50} in mice of approximately 2600 mg/kg. MMF was moderately toxic by skin absorption in the pregnant rabbit with an ALD of 1500 mg/kg and exhibited very low toxicity by skin absorption in the pregnant rat with an ALD of 11,000 mg/kg. The ALD studies were conducted in pregnant rats and rabbits as the dose selection portion of an embryotoxicity study. MMF was irritating to

rabbit eyes. No information was available on dermal irritation or sensitization. **No further acute toxicity testing is recommended for MMF.**

Table 5: Mammalian Acute Toxicity

Oral LD_{50}	4000 – 7077 mg/kg (rat) 2600 mg/kg (mice)
4-hour Inhalation ALC	> 10.76 mg/L (rat)
Dermal ALD	11,000 (rat) 1500 (rabbit)
Eye Irritation	Irritating

Repeated Dose Toxicity

The primary target organ in repeated dose studies appears to be the liver for both MMF and DMF. These effects appear at similar doses/exposures to the 2 chemicals. In a two-week inhalation study with MMF, no adverse effects were seen in rats exposed to 50 ppm. Higher concentrations (132 and/or 402 ppm) produced compound-related biochemical and microscopic pathology changes in the liver. Longer term repeated dose studies of MMF were not available; however, data were available on the structurally similar compound, DMF. In a two-week inhalation study in rats with DMF, increased liver weights were observed at 91 ppm. In a 90-day inhalation study with DMF, evidence of hepatocellular injury was seen as early as day 4 on increases in the activities of liver-specific enzymes at concentrations of 200 ppm and above. Relative liver weights were increased in males at 100 ppm and above and in females at 50 ppm and above. Pathologic changes of the liver (minimal to moderate centrilobular hepatocellular necrosis) were observed at 400 ppm and above. **No further repeated dose toxicity testing is recommended.**

Table 6: Repeated Dose Toxicity

	MMF	DMF
Two-Week Inhalation Study	NOEL = 50 ppm Pathologic changes in the liver and increased liver weights at 132 and/or 402 ppm.	NOEL < 91 ppm Increased liver weights were observed at 91 ppm.
90-Day Inhalation Study	No Data	NOEL< 50 ppm Biochemical changes in liver enzymes at 200 ppm and above in rats. Increased relative liver weights at 50 ppm and above in rats and mice. Pathologic changes in the liver at 400 ppm and above in rats and 50 ppm and above in mice.

Developmental Toxicity

For both MMF and DMF, the fetus appears to have the same sensitivity to the test chemicals as the maternal animal.

MMF did not produce developmental effects at maternally non-toxic doses when given by inhalation to rats (Rickard et al., 1995), dermally to rats (Stula and Krauss, 1977), and orally to rats (Kelich et al., 1995; Merkle and Zeller, 1980) and rabbits (Kelich et al., 1995). Developmental effects were observed in mice when treated with MMF orally and dermally (Roll and Baer, 1967). The quality of these studies vary, ranging from scientifically rigorous study design and thorough reporting (Kelich et al., 1995; Rickard et al., 1995), to studies with little study design and reporting details (e.g. maternal toxicity not reported or differential toxicity reported with limited study information) (see Section 5.3 for complete listing of studies). In studies in which both maternal and fetal effects were carefully examined (Kelich et al., 1995; Rickard et al., 1995), the effects of MMF appeared at the same dose levels in maternal and fetal animals. In the inhalation study in rats (Rickard et al., 1995), maternal lethality and toxicity was demonstrated at 150 ppm MMF and maternal toxicity remained evident as mild respiratory distress in the 50 ppm treated dams. Decreased fetal weight and fetal malformations and variations were observed at 150 ppm. Developmental toxicity, expressed as slight depression in fetal weight, was evident at 50 ppm. The NOEL for both the dam and the fetus was 15 ppm. In an oral study in rats and rabbits (Kelich et al., 1995), maternal toxicity was evidenced as decreased body weight and food consumption at 75 mg/kg in rats and 50 mg/kg in rabbits. Developmental toxicity was evidenced by reduced fetal viability, reduced fetal weight, and fetal malformations at 75 mg/kg in rats and 50 mg/kg in rabbits. The NOEL for maternal and fetal toxicity was 10 mg/kg in both rats and rabbits.

The weight of evidence from testing which has been conducted in rats and rabbits by inhalation, oral, and dermal routes of exposure shows that DMF affects the embryo/fetus only under conditions which will affect the maternal animal. Rats exposed by inhalation to either 18 or 172 ppm during gestation showed no structural changes. Both maternal and fetal weights were reduced at 172 ppm (Kimmerle and Machener, 1975). Similarly, no teratogenic effects were seen in rats inhaling either 30 or 300 ppm during gestation with weights affected in both maternal and fetal rats at 300 ppm (Lewis et al., 1992). Oral studies in rats showed maternal toxicity at doses of 100 mg/kg or greater along with fetal toxicity at the same doses. No malformations were seen and the fetal effect consisted of weight depressions and skeletal developmental delays (Saillenfait et al., 1997). DMF given orally to rabbits produced both maternal and fetal effects with fetal anomalies being produced at doses that had little maternal effect. The authors report a fetal NOEL of 44.1 mg/kg and a maternal NOEL of 65 mg/kg (Merkle and Zeller, 1980). Studies in other species and those involving dermal exposure supports the hypothesis that the maternal and fetal animals are equally sensitive to the toxic effects of DMF (Kennedy, 1986; 2001). **No further developmental toxicity testing is recommended.**

Table 7: Developmental Toxicity

	MMF	**DMF**
Inhalation Study in rats	Maternal and fetal NOEL = 15 ppm	Maternal and fetal NOEL = 18 ppm Maternal and fetal NOEL = 30 ppm
Oral Study in rats	Maternal and fetal NOEL = 10 mg/kg	Maternal and fetal NOEL = 50 mg/kg
Oral Study in rabbits	Maternal and fetal NOEL = 10 mg/kg	Maternal NOEL = 65 mg/kg Fetal NOEL = 44.1 mg/kg

Reproductive Toxicity

No formal reproductive toxicity studies have been conducted on MMF. Data were available on the structurally similar compound, DMF. In a continuous breeding study in which mice were exposed to either 1000, 4000, or 7000 ppm of DMF in their drinking water, a decrease in fertility (reflected by a decrease in pups born alive and in live litter size) was seen at 4000 and 7000 ppm. Liver damage was produced in all parental animals (1000 to 7000 ppm) and body weight gains were affected at 4000 and 7000 ppm. Decreased fertility paralleled the parental sensitivity observed at 4000 and 7000 ppm. Decreased pup weight was observed in the F2 pups at 1000 ppm. In a 90-day inhalation study conducted in rats and mice, relative testes weights were increased at 400 ppm and above in the rats; however, no microscopic findings or adverse effects on sperm density or motility were observed in rats or mice. DMF is not considered a unique reproductive toxicant (any reproductive effects have been shown to occur at higher doses/exposures than hepatotoxic effects). Based on its structural similarity and its similar toxicity profile for developmental and repeated dose toxicity, we expect MMF to not be a reproductive toxin. **No reproductive testing is recommended for MMF.**

Table 8: Reproductive Toxicity

	MMF	DMF
Reproductive Toxicity	No Data	Not a unique reproductive toxin* in a continuous breeding study
* = reproductive effects have been shown to occur at the same doses/exposures as hepatotoxic effects		

Genetic Toxicity

MMF was not mutagenic in *Escherichia coli*. No data on the clastogenicity of MMF are available. However the genetic toxicity of the analog, DMF, has been extensively investigated. A review of the available literature indicates that although some positive findings have been observed, DMF does not induce chromosome aberrations or gene mutations in most of the systems tested. In *in vitro* bacterial mutation assays, 33/37 tests with DMF produced negative results. DMF was also negative in 14/14 unscheduled DNA synthesis assays (*in vitro*), negative in 19/22 clastogenicity assays (*in vitro*), negative in 8/9 *in vivo* micronucleus assays, negative in 11/11 *in vivo* dominant lethal tests, and negative in 17/17 other *in vivo* genetic toxicity assays. The weight of evidence suggests that DMF and, by analogy, MMF are not genotoxic. **No further genotoxicity testing is recommended for MMF.**

Table 9: GeneticToxicity

	MMF	DMF
Mutagenicity	Not mutagenic	Not mutagenic
Clastogenicity	No Data	Not clastogenic

Metabolism

The pathways for biotransformation of N,N-dimethylformamide (DMF) and N-methylformamide (MMF) have been the subject of extensive investigation for over 30 years. The primary driver behind this effort has been the realization that the toxicity of these compounds is intrinsically related to their metabolism, both quantitatively and qualitatively. The primary pathways for biotransformation of DMF and MMF are illustrated in Figure 1. The major pathway for primary metabolism of DMF in all species studies, including humans, is the P450-mediated oxidation of one of the *N*-methyl moieties to form the stable carbinolamide *N*-(hydroxymethyl)-*N*-methylformamide (HMMF, pathway 1) (Gescher, 1993; Van den Bulcke et al., 1994; Hundley et al., 1993a; 1993b; Lareo and Perbelline, 1995). Several lines of evidence indicate that CYP2E1 is the major catalyst for this reaction (Mráz et al., 1993). HMMF is readily excreted in the urine, accounting for approximately 50% of the administered dose of DMF in rats and 22% of the dose in human volunteers exposed by inhalation (Van den Bulcke et al., 1994; Mráz and Nohova, 1992). An alternative pathway for biotransformation of DMF involves formal demethylation to yield MMF (pathway 2). Demethylation of DMF to MMF is thought to occur both directly (Scailteur and Lauwerys, 1984) and via hydrolysis of HMMF (pathway 2a) (Van den Bulcke et al., 1994).

MMF thus formed is further metabolized by two distinct pathways. The first pathway involves hydroxylation of the remaining methyl group to form *N*-(hydroxymethyl)formamide (HMF, pathway 4), analogous to the oxidation of DMF to HMMF (Kestell et al., 1985; Tupil and Timbrell, 1988). Urinary HMF accounted for approximately 3-6% and 7-9% of the administered dose of MMF in rats and mice, respectively. HMF has not been quantified following administration of DMF, but approximately 13% of the administered dose of DMF in a study involving human volunteers was recovered in the urine as formamide, most of which was thought to result from thermal decomposition of HMF in the analytical system (Mráz and Nohova, 1992). By analogy with HMMF, a small amount of MMF is thought to undergo demethylation to formamide, presumable involving the intermediacy of HMF (pathway 5) (Kestell et al., 1985). The second pathway for further metabolism of MMF is oxidation of the formyl carbon (pathway 3), leading to formation of a highly reactive intermediate thought to play a pivotal role in toxicity of both DMF and MMF. This reaction appears to be catalyzed primarily, if not exclusively, by CYP2E1 (Mráz et al., 1993; Chieli et al., 1995). The existence of this intermediate has been inferred from the detection of *S*-(*N*-methylcarbamoyl)glutathione (SMG) and *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine (AMMC). The metabolite generated by oxidation of the formyl moiety has not been unequivocally identified, but methyisocyanate has been proposed as a likely candidate, as shown in figure 1 (Gescher, 1993). However, other intermediates such as *N*-methylcarbamic acid have not been ruled out (Gescher, 1993; Kestell et al., 1985). There is some evidence to suggest that HMMF may also be oxidized at the formyl carbon, generating the reactive intermediate following hydrolysis of the *N*-hydroxymethyl group (pathway 3), though this route can at most account for ~5% of the total carbamoylating intermediate (Van den Bulcke et al., 1994; Mráz et al., 1993). As indicated in Figure 1, the reactive intermediate can participate in covalent binding to proteins and transcarbamoylation reactions, and is thought to be directly responsible for hepatotoxicity of both DMF and MMF. The carbamoylating intermediate can react with cellular glutathione (GSH) to yield SMG (pathway 6), which has been detected as a biliary metabolite of DMF and MMF (Gescher, 1993). SMG proceeds through the mercapturic acid pathway (pathway 7), and is eventually excreted in the urine as AMMC. Quantitation of urinary AMMC has been proposed as a useful biomarker for occupational exposure to DMF (Lareo and Perbellini, 1995; Sakai et al., 1995). Human volunteers exposed to 30 mg/m^3 DMF excreted approximately 13% of the dose as AMMC (Mráz and Nohova, 1992). As indicated previously, the formation of SMG is reversible, and this metabolite can be hydrolyzed to form methylamine, as could AMMC. Alternatively, methylamine could be formed by direct hydrolysis, or decomposition of the carbamoylating intermediate (pathway 8). The involvement of the reactive intermediate in methylamine formation has been inferred from the prominent kinetic deuterium isotope effect on methylamine formation observed following carbamoyl-^{2}H-MMF in mice (Threadgill et al., 1987).

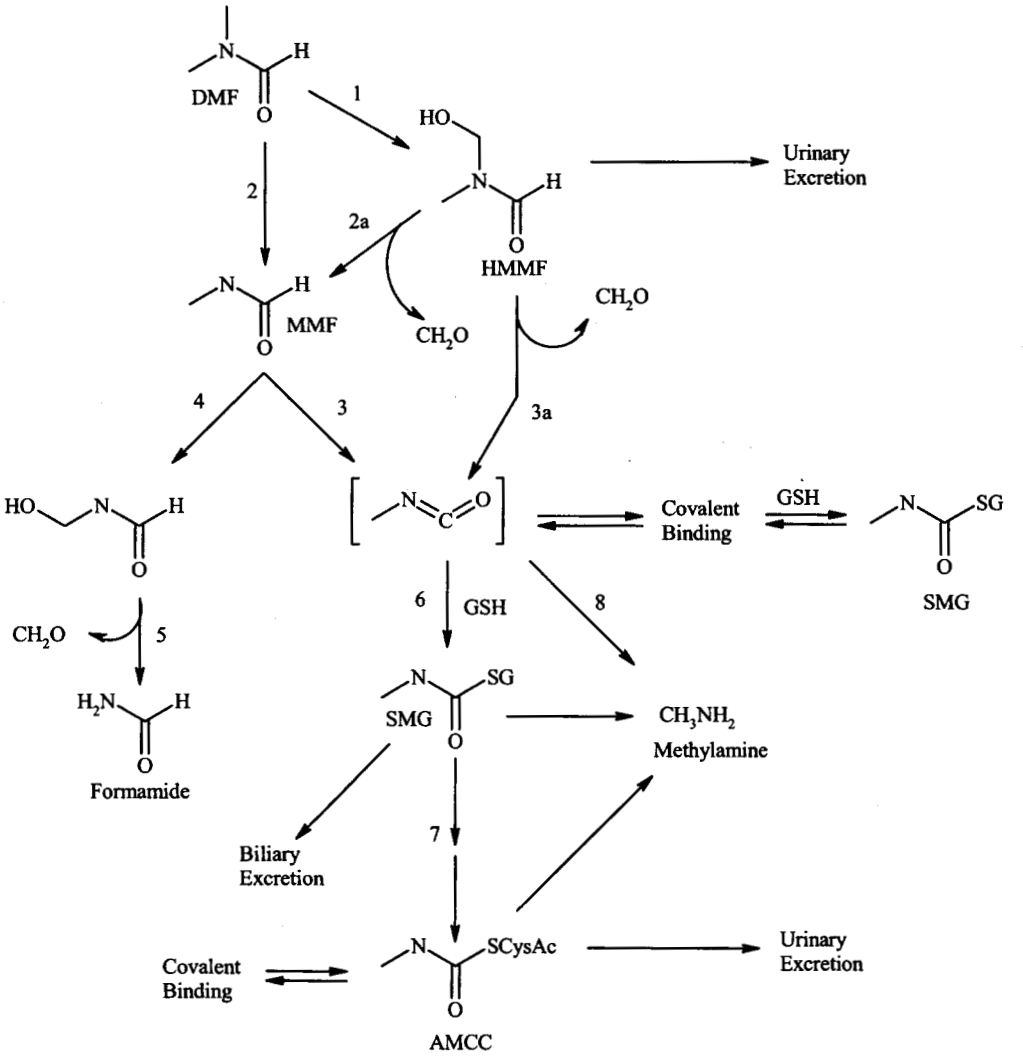
Pharmacokinetic studies of DMF in various species have demonstrated that the area under the plasma concentration vs. time curves (AUC) for DMF increases in the order monkey < mouse < rat following inhalation exposure to comparable concentrations (Hundley et al., 1993a; 1993b). Further, the AUC was found to increase out of proportion with increased exposure concentration, suggesting saturation of DMF metabolism in all thee species. In all species, the major urinary metabolite was HMMF, followed by DMF and MMF (Hundley et al., 1993a; 1993b). AMMC was not measured in these studies. Comparative excretion studies of DMF in rodents and

humans have demonstrated significant quantitative differences in urinary metabolite profiles following i.p. (rodents) or inhalation (humans) exposure (Mráz et al., 1989). In this study, only minute concentration of DMF were detected. Differences in the proportion of the dose excreted unchanged between this and other (Hundley et al., 1993a) studies may be due in part to differences in the route of administration. Excretion of HMMF was greatest in rats (36.8%, followed by humans (25.9%) and mice (8.4%). Formamide (representing formamide + HMF) accounted for 23-38% of the dose in rodents and approximately 14% of the dose in humans. Approximately 1.6-5.2% of the dose was excreted at AMMC in rodents, while humans excreted an average of 14.2% of the dose as AMMC. Following exposure to DMF, excretion of HMMF and MMF are rapid, while excretion of AMMC is delayed, particularly in humans (Mráz and Nohova, 1992). In rodents, hepatotoxicity of DMF is delayed at higher doses compared to lower doses, and this is though to result from the inhibition of metabolism of MMF to reactive species by DMF. Consistent with this idea is the finding that the K_M value for metabolism of MMF to SMG is approximately 20 fold higher than the K_M for metabolism of DMF to HMMF (Mráz et al., 1993). Further, both the metabolism and the hepatotoxicity of MMF were delayed in rats treated simultaneously with DMF (Van den Bulcke et al., 1994; Lundberg et al., 1983). In addition, DMF induces its own metabolism in mice and rats, with lower plasma AUCs observed following repeated exposure compared to single exposure (Hundley et al., 1993a). This effect was not observed in monkeys exposed repeatedly to DMF by inhalation (Hundley et al., 1993b). However, there was a shift in the balance of urinary metabolites in male monkeys, suggesting slightly greater metabolism of DMF to HNNF and MMF following repeated exposure.

In mice, the plasma half-life of MMF administered by *i.p.* injection was approximately 3.6 hours (Gescher et al., 1982). When MMF was administered to rats and mice, a significant fraction of the administered dose was excreted as unchanged MMF in the urine (Tupil and Timbrell, 1988). Excretion of unchanged MMF was greater in rats (23-40%) than in mice (10-12%). In mice, methylamine was the major urinary metabolite (~30% of the dose), whereas this was a comparatively minor metabolite in rats (<10%). Approximately 3-6% of the administered dose was excreted as AMMC in both species. In mice, greater than 90% of the AMMC was excreted within the first 24 hours after dosing with MMF. However, in rats, only about 35% of the total AMMC was excreted in the first 24 hours, with the remainder excreted between 24 and 48 hours. These data are consistent with the generally more rapid metabolism of MMF and greater severity of hepatotoxicity in mice compared to rats, and suggest that the rat may be a more appropriate model for MMF toxicity than the mouse.

Overall, the pathways of metabolism for DMF and MMF are qualitatively similar. Hepatotoxicity of both compounds is thought to be mediated by a reactive carbamoylating intermediate formed by oxidation of the formyl carbon of MMF. Consequently, the hepatotoxicity of the two compounds is qualitatively similar. However, due to inhibition of formyl oxidation step by DMF itself, the hepatotoxicity of the latter compound is delayed with respect to time of exposure.

Figure 1. Metabolic Pathways for Dimethylformamide (DMF) and N-Methylformamide (MMF)



Human Exposure

The predominant use (greater than 95%) of monomethylformamide (MMF) is as a DuPont-limited intermediate. Less than 5% of DuPont's production is sold to external customers. These customers, located in Europe and Japan, use MMF for industrial purposes only: as a solvent in electronics manufacture and as a solvent for chemical synthesis of resins. Personal protective equipment and ventilation are used at these sites to minimize worker exposure.

For the major use, MMF is manufactured at one DuPont plant and is shipped by railcar to another DuPont facility for use as a raw material. MMF is catalytically converted *in-situ* to

another substance, which is then further reacted to form downstream products at the site. The manufacture and use are closed system operations. The only potential human exposure occurs during loading and unloading.

Monomethylformamide is made on a campaign basis twice a year. Monomethylformamide is produced in a closed system and is hard piped to loading spots. Flex hose is connected to the liquid valve on the tank car and the liquid is fed through an induction pipe to the bottom of car. The dome lid is kept down except during periods when operators are performing level checks and collecting samples. Off-gases associated with the MMF process are vented to a flare stack. MMF is shipped by rail to the DuPont use site and is unloaded into a storage tank and is consumed throughout the year. At the DuPont use site, MMF is handled in a closed system except when piping connections are made during the unloading process. A nitrogen pad is kept on the railcar during the unloading process and any vented material goes directly to an incinerator. All transfers from the storage tank and subsequent consumption in the reactor occur under closed system conditions, without exposure to workers.

At the DuPont manufacturing site, potential for employee exposure is greatest when operators perform periodic checks on top of MMF railcars to inspect the dome, do level checks, and collect samples. Operators wear appropriate personal protective equipment (PPE) to protect themselves from liquid and vapor contact while on the railcar. PPE consists of a positive pressure air supplied respirator, Nomex coveralls, and neoprene gloves. Safety showers, eyewash stations, and self-contained breathing apparatus (SCBA) are available in close proximity to the operations area. Chemical splash goggles, Nomex coveralls and neoprene gloves are required by all personnel for patrol-type work during the production of MMF.

At the DuPont use site, employee potential for exposure occurs only during unloading operations. When making connections to lines containing MMF, chemical suit, boots, NIOSH approved hood respirator, and gloves are worn. Chemical gloves, chemical jacket, chemical splash goggles, and faceshield are required for sampling activities involving MMF.

The DuPont Acceptable Exposure Limit (AEL) for monomethylformamide is 2 ppm as an 8- and 12-hour TWA (time-weighted average). Air monitoring has been conducted on monomethylformamide and all measured concentrations are well below the AEL. Results are shown in the table below:

(June 15, 2006)

Exposure Data:

Job Sampled	No. of Results	Average (ppm)	Minimum (ppm)	Maximum (ppm)
DuPont Manufacturing Site Operators (full shift)	**18**	**<0.1**	**<0.1**	**<0.1**
DuPont Manufacturing Site Loaders	**18**	**0.162**	**<0.1**	**0.6**
DuPont Use Site Unloaders (full shift)	**13**		**All <0.1**	

Conclusion

Adequate data are available to address the required HPV endpoints. A substantial body of data exists for MMF *per se*. Where data are lacking on MMF, reliable data are available for the close analog, DMF. The use of DMF data to supplement the existing mammalian toxicity data for MMF is supported by the close similarity in molecular structure, similarity in physical/chemical properties, and the similarity in toxicity observed where data for both substances are available for comparison. Further strong support for use of DMF as an analog for MMF is provided by the extensive understanding of the metabolic fate of DMF and MMF, and the fact that MMF is one of the products of metabolism of DMF. The use of DMF as an analog to MMF is consistent with the Agency's directive to HPV participants to maximize the use of scientifically appropriate data for related chemicals. Although some differences between MMF and DMF may be expected, we believe these differences to be minimal and insufficient to warrant additional animal testing.

TEST PLAN FOR N-METHYL FORMAMIDE

N-Methyl formamide CAS No. 123-39-7	Data Available	Data Acceptable	Testing Required
Study	**Y/N**	**Y/N**	**Y/N**
PHYSICAL/CHEMICAL CHARACTERISTICS			
Melting Point	Y	Y	N
Boiling Point	Y	Y	N
Vapor Pressure	Y	Y	N
Partition Coefficient	Y	Y	N
Water Solubility	Y	Y	N
ENVIRONMENTAL FATE			
Photodegradation	Y	Y	N
Stability in Water	Y	Y	N
Transport (Fugacity)	Y	Y	N
Biodegradation	Y	Y	N
ECOTOXICITY			
Acute Toxicity to Fish	Y	Y	N
Acute Toxicity to Invertebrates	Y	Y	N
Acute Toxicity to Aquatic Plants	Y	Y	N
MAMMALIAN TOXICITY			
Acute Toxicity	Y	Y	N
Repeated Dose Toxicity	Y*	Y	N
Developmental Toxicity	Y	Y	N
Reproductive Toxicity	Y*	Y	N
Genetic Toxicity Gene Mutations	Y*	Y	N
Genetic Toxicity Chromosomal Aberrations	Y*	Y	N
Y = yes; N= no; NR = not required * = Data are available on an analog chemical.			

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I U C L I D

Data Set

Existing Chemical	:	ID: 123-39-7
CAS No.	:	123-39-7
EINECS Name	:	N-methylformamide
EC No.	:	204-624-6
TSCA Name	:	Formamide, N-methyl-
Molecular Formula	:	C2H5NO

Producer related part

Company	:	E. I. du Pont de Nemours and Company
Creation date	:	02.02.2006

Substance related part

Company	:	E. I. du Pont de Nemours and Company
Creation date	:	02.02.2006
Status	:	
Memo	:	
Printing date	:	15.06.2006
Revision date	:	
Date of last update	:	24.04.2006
Number of pages	:	84
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

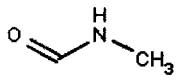
1.1.0 SUBSTANCE IDENTIFICATION

IUPAC Name : N-methyl formamide
Smiles Code : O=CNC
Molecular formula : C2H5NO
Molecular weight : 59.07
Petrol class :

02.02.2006

1.1.1 GENERAL SUBSTANCE INFORMATION

Attached document : mmf.bmp



02.02.2006

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

EK 7011

02.02.2006

Formamide, N-methyl-

02.02.2006

Formic acid amide, N-methyl-

02.02.2006

Methylformamide

02.02.2006

Monomethylformamide (MMF)

02.02.2006

N-Methylformamide (NMF)

02.02.2006

N-Methylformic acid amide

02.02.2006

N-Methylformimidic acid

02.02.2006

N-Monomethylformamide

02.02.2006

NCS 3051

02.02.2006

X 188

02.02.2006

1.3 IMPURITIES

1.4 ADDITIVES

1.5 TOTAL QUANTITY

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.6.3 PACKAGING

1.7 USE PATTERN

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

Type of limit : other: DuPont Acceptable Exposure Limit (AEL)
Limit value : 2 other: ppm

Remark : AEL is listed as an 8- and 12-hour TWA and the AEL includes a skin notation.

02.02.2006 (49)

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

1.8.4 MAJOR ACCIDENT HAZARDS

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

1.11 ADDITIONAL REMARKS

Remark : Existing published and unpublished data were collected and scientifically evaluated to determine the best possible study or studies to be summarized for each required endpoint. In the spirit of this voluntary program, other data of equal or lesser quality are not summarized, but are listed as related references at the end of each appropriate section, with a statement to reflect the reason why these studies were not summarized.

02.02.2006

1.12 LAST LITERATURE SEARCH

1.13 REVIEWS

2.1 MELTING POINT

Value	:	-3.8 °C
Sublimation	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Reliability: Not assignable because limited study information was available.
02.02.2006		(106)
Remark	:	Additional References for Melting Point:
02.02.2006		(22) (49) (160) (188)
Remark	:	Supporting Data for Dimethylformamide (DMF)
06.02.2006		
Value	:	-61 °C
Sublimation	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	other TS
Remark	:	Reliability: Not assignable because limited study information was available.
Test substance	:	Dimethylformamide (DMF)
06.02.2006		(18)

2.2 BOILING POINT

Value	:	199.5 °C at
Decomposition	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Reliability: Not assignable because limited study information was available.
02.02.2006		(106)
Remark	:	Additional References for Boiling Point:
02.02.2006		(22) (49) (61) (103) (188)
Remark	:	Supporting Data for Dimethylformamide (DMF)
06.02.2006		
Value	:	152.5 - 153.5 °C at
Decomposition	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	other TS

Remark : Reliability: Not assignable because limited study information was available.
Test substance : Dimethylformamide (DMF)
06.02.2006 (18)

2.3 DENSITY

Type : density
Value : .9961 g/cm³ at 25 °C
Method :
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : Reliability: Not assignable because limited study information was available.
02.02.2006 (22)

Remark : Additional References for Density:
02.02.2006 (49) (103) (106) (188)

Remark : Supporting Data for Dimethylformamide (DMF)
06.02.2006

Type : density
Value : .95 g/cm³ at 25 °C
Method :
Year :
GLP : no data
Test substance : other TS

Remark : Reliability: Not assignable because limited study information was available.
Test substance : Dimethylformamide (DMF)
06.02.2006 (18)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : .337 hPa at 25 °C
Decomposition :
Method :
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : Reliability: Not assignable because limited study information was available.
Result : 0.253 mm Hg (converted to 0.337 hPa)
02.02.2006 (30)

Remark : Additional References for Vapor Pressure:
02.02.2006 (49) (160) (188)

Remark : Supporting Data for Dimethylformamide (DMF)

06.02.2006

Value	:	4.7988 hPa at 25 °C
Decomposition	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	other TS
Remark	:	Reliability: Not assignable because limited study information was available.
Result	:	3.6 mm Hg (converted to 4.7988 hPa)
Test substance	:	Dimethylformamide (DMF)

06.02.2006 (81)

2.5 PARTITION COEFFICIENT

Partition coefficient	:	octanol-water
Log pow	:	-.97 at °C
pH value	:	
Method	:	
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Reliability: Not assignable because limited study information was available.

02.02.2006 (64)

Partition coefficient	:	octanol-water
Log pow	:	-1.14 at 25 °C
pH value	:	
Method	:	
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Modeled. KOWWIN, v. 1.67, module of EPIWIN 3.11 (Syracuse Research Corporation). KOWWIN uses "fragment constant" methodologies to predict log P. In a "fragment constant" method, a structure is divided into fragments (atom or larger functional groups) and coefficient values of each fragment or group are summed together to yield the log P estimate.
Remark	:	Reliability: Estimated value based on accepted model.

02.02.2006 (125)

Remark : Additional Reference for Partition Coefficient (log Kow):

02.02.2006 (102)

Remark : Supporting Data for Dimethylformamide (DMF)

06.02.2006

Partition coefficient	:	octanol-water
Log pow	:	-.85 at 25 °C
pH value	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	other TS
Method	:	Mean value of 3 measurements.

Remark	:	Reliability: Valid with restrictions (reliability given in the SIDS Dossier).
Test substance	:	Dimethylformamide (DMF)

06.02.2006 (13)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	at °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4

Remark	:	Reliability: Not assignable because limited study information was available.
Result	:	1.0x10E6 mg/L at 25°C

02.02.2006 (69)

Remark	:	Additional References for Water Solubility:

02.02.2006 (49) (103) (106) (160) (188) (190)

Remark	:	Supporting Data for Dimethylformamide (DMF)

06.02.2006

Solubility in	:	Water
Value	:	200 g/l at 20 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	other TS

Remark	:	Reliability: Not assignable because limited study information was available.
Test substance	:	Dimethylformamide (DMF)

06.02.2006 (16)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

Value	:	119 °C

Type	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Reliability: Not assignable because limited study information was available.
02.02.2006		(49)
Remark	:	Additional References for Flash Point:
02.02.2006		(103) (174)
Remark	:	Supporting Data for Dimethylformamide (DMF)
06.02.2006		
Value	:	57.5 °C
Type	:	other: Closed Cup, DIN 51 755
Method	:	
Year	:	
GLP	:	no data
Test substance	:	other TS
Remark	:	Reliability: Valid with restrictions (reliability given in the SIDS Dossier).
Test substance	:	Dimethylformamide (DMF)
06.02.2006		(10)

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

Method	:	
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Reliability: Not assignable because limited study information was available.
Result	:	Autoignition: 323°C
		Explosive limits: 1.8-19.7%
02.02.2006		(49)
Remark	:	Supporting Data for Dimethylformamide (DMF)
06.02.2006		
Method	:	other: DIN 51 794
Year	:	
GLP	:	no data
Test substance	:	other TS
Remark	:	Reliability: Valid with restrictions (reliability given in the SIDS Dossier).
Result	:	Autoignition: 410°C
Test substance	:	Dimethylformamide (DMF)
06.02.2006		(10)

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
Deg. product	:	
Method	:	other (calculated): Modeling and inspection of adsorption spectrum
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Reliability: Estimated value based on accepted model.
Result	:	Direct Photolysis: Not expected due to lack of adsorption at equal to or greater than 290 nm.

06.02.2006 (65)

Type	:	air
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	
Rate constant	:	cm^3/(molecule*sec)
Degradation	:	% after
Deg. product	:	
Method	:	other (calculated): Modeling and inspection of adsorption spectrum
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Indirect Photolysis: AOPWIN, v. 1.91 module of EPIWIN 3.11.
Remark	:	Reliability: Estimated value based on accepted model.
Result	:	Indirect Photolysis: 57 hour half-life due to OH radical reactions.

02.02.2006 (124)

Remark	:	Data from this additional source were not summarized because insufficient study information was available.

02.02.2006 (135)

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	at °C
t1/2 pH7	:	at °C
t1/2 pH9	:	at °C
Deg. product	:	
Method	:	
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Reliability: Estimated value based on accepted model.
Result	:	N-Methylformamide is not expected to undergo hydrolysis in the environment since amides hydrolyze very slowly under environmental

conditions (Mabey and Mill, 1978).

02.02.2006 (109)

Type	:	abiotic
t1/2 pH4	:	at °C
t1/2 pH7	:	at °C
t1/2 pH9	:	at °C
Deg. product	:	
Method	:	other (calculated)
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Modeled. HYDROWIN, v. 1.67 module of EPIWIN v3.11 (Syracuse Research Corporation). HYDROWIN estimates aqueous hydrolysis rate constants for the following chemical classes: esters, carbamates, epoxides, halomethanes and selected alkyl halides. HYDROWIN estimates acid- and base-catalyzed rate constants; it does NOT estimate neutral hydrolysis rate constants. The prediction methodology was developed for the U.S. Environmental Protection Agency and is outlined in Mill et al., 1987. Half-life prediction is based on the observation of an amide group on SMILES atom 2, not on a QSAR equation. "Compound has an amide group; C=O located at SMILES atom #: 2".
Remark	:	Reliability: Estimated value based on accepted model.
Result	:	Half-life: Hydrolysis Rate Extremely Slow or t1/2 > 1 Year

02.02.2006 (66) (128)

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	fugacity model level III
Media	:	other: Air, water, soil, and sediment
Air	:	% (Fugacity Model Level I)
Water	:	% (Fugacity Model Level I)
Soil	:	% (Fugacity Model Level I)
Biota	:	% (Fugacity Model Level II/III)
Soil	:	% (Fugacity Model Level II/III)
Method	:	other: Modeled
Year	:	
Method	:	Modeled. Henry's Law Constant - HENRYWIN v. 3.10 module of EPIWIN v3.11 (Syracuse Research Corporation). Henry's Law Constant (HLC) is estimated by two separate methods that yield two separate estimates (Hine and Mookerjee, 1975; Meylan and Howard, 1991). The first method is the bond contribution method and the second is the group contribution method. The bond contribution method is able to estimate many more types of structures; however, the group method estimate is usually preferred (but

not always) when all fragment values are available.

Koc - Calculated from Kow by the Mackay Level III fugacity model incorporated into EPIWIN v3.11 (Syracuse Research Corporation).

Environmental Distribution - Mackay Level III fugacity model, in EPIWIN v3.11 (Syracuse Research Corporation). Emissions (1000 kg/hr) to air, water, and soil compartments.

Fugacity - The methodology and programming for the Level III fugacity model incorporated into EPIWIN v3.11 (Syracuse Research Corporation) were developed by Dr. Donald MacKay and coworkers and are detailed in Mackay, 1991; Mackay et al., 1996a, 1996b.

Remark : Reliability: Estimated value based on accepted model.

Result : Distributions:

Compartment	% of total distribution*	½ life (advection + reaction)
Air	0.43	57
Water	39.7	360
Soil	59.8	720
Sediment	0.073	3240

* - based on standard emission scenario:1000 kg/h each for air, water and soil

Adsorption Coefficient: Koc = 0.0439

Desorption: No Data

Test condition : Level III Inputs:

SMILES Code: O=CNC
Molecular Wt: 59.07
Henry's LC: 1.97x10E-8 atm-m3/mole (Henry database)
Vapor Press : 0.253 mm Hg (user-entered)
Log Kow: -0.97 (Kowwin program)
Soil Koc: 0.0439 (calc by model)

15.02.2006 (68) (111) (112) (113) (123)

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type : aerobic
Inoculum : activated sludge, domestic
Contact time :
Degradation : 80 (±) % after 28 day(s)
Result : readily biodegradable
Deg. product :
Method : OECD Guide-line 301 B "Ready Biodegradability: Modified Sturm Test (CO_2 evolution)"
Year : 2006
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Method : MMF was tested for ready biodegradability using the 28-day CO2 evolution test for "Ready Biodegradation" according to OECD guideline 301B in the version dated July 17, 1992. This test is also known as the Modified Sturm Test.

A predetermined amount of the test substance was added to an inorganic medium (mineral nutrient solution) providing 20 mg C per liter. The solution was inoculated with a small number of microorganisms from a mixed population and kept well aerated using humidified CO2-free air. The biological system used was secondary activated sludge from the West Chester Pennsylvania Publicly-Owned Treatment Works (POTW). At 0, 1, 4, 6, 8, 11, 13, 18, 21, 28, and 29 days, the carbon dioxide trapped in the barium hydroxide was measured by titration of the residual hydroxide. The amount of CO2 produced from the test substance (corrected for that derived from the blank inoculum) was expressed as a percentage of the total CO2 that the test material could have theoretically produced based on carbon composition (ThO2). Test substances giving a result greater than 60% yield of CO2 (within 28 days) are regarded as readily biodegradable. This level must be reached within 10 days of biodegradation exceeding 10% within the 28-day test period.

The control substance used was sodium benzoate and was used to check the activity of the inoculum. The control substance needed to give a yield of > 60% of the theoretical total CO2 within 14 days for the test to be considered valid.

No test vehicle was used. The control and test substances were added directly to the test solutions.

Remark : Reliability: High because a scientifically defensible or guideline method was used.

Result : MMF reached a maximum biodegradability of 80% by day 28. Greater than 60% biodegradability was reached within 10 days of exceeding 10% biodegradation.

In the toxicity control, which includes both the test substance and control substance in the same flask, the substances yielded >25% biodegradation within 6 days. The control substance attained a biodegradation level of 61% by day 6 thus the test was considered valid.

MMF was considered "readily biodegradable." MMF was not inhibitory to microorganisms in the inoculum.

Test substance : N-methylformamide, purity 99.8%

24.04.2006 (50)

Deg. product :
Method : other
Year : 1982
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Zahn-Wellens Inherent Biodegradability test: 400 mg/L initial test compound concentration
An industrial activated sludge inoculum was used.

Remark : Reliability: Not assignable because limited study information was available.

Result : 4% ThBOD in 3 hours
98% ThBOD in 3 days
100% ThBOD in 7 days

24.04.2006 (11)

Type : aerobic
Inoculum :

Deg. product	:	
Method	:	other: Modeled
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Modeled. BIOWIN, v. 4.01 module of EPIWIN v3.11 (Syracuse Research Corporation). BIOWIN estimates the probability for the rapid aerobic biodegradation of an organic chemical in the presence of mixed populations of environmental microorganisms. Estimates are based upon fragment constants that were developed using multiple linear and non-linear regression analyses.
Remark	:	Reliability: Estimated value based on accepted model.
Result	:	Linear Model Prediction: 0.86 [Degrades Fast] Non-Linear Model Prediction: 0.99 [Degrades Fast] Ultimate Biodegradation Timeframe: 3.09 Weeks Primary Biodegradation Timeframe: 3.91 Days MITI Linear Model Prediction: 0.76 [Readily Degradable] MITI Non-Linear Model Prediction: 0.87 [Readily Degradable] Breakdown Products: No Data

24.04.2006 (19) (70) (71) (186)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

BCF	:	3
Elimination	:	
Method	:	other: Modeled
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Modeled. BCFWIN v. 2.15 module of EPIWIN v3.11 (Syracuse Research Corporation). BCFWIN estimates the bioconcentration factor (BCF) of an organic compound using the compound's log octanol-water partition coefficient (Kow) with correction factors based on molecular fragments.
Remark	:	Reliability: Estimated value based on accepted model.

14.02.2006 (127)

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	static
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	> 10000
Method	:	other
Year	:	1985
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Method : No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

The test material, in original form, was introduced into commercial, glass, rectangular 21-L aquaria and diluted with laboratory well water to yield the desired exposure concentrations in 15 L final volumes. An identical vessel containing only laboratory well water was designated as the control.

Ten fathead minnows, with a 2.4 cm mean standard length and 0.15 g mean wet weight, were randomly assigned to each test vessel. The fish were not fed for 48 hours prior to nor during the exposure. The nominal test solutions (0, 1, 100, 1000, 10,000 mg/L) were not aerated. Temperature was maintained at 22°C. Photoperiod was maintained at 16 hours light: 8 hours dark.

Mortality counts and observations were made every 24 hours during the 96-hour exposure period.

Dissolved oxygen and pH were measured in the control and 1, 1000, and 10,000 mg/L test concentrations at the beginning of the test, at 48-hours, and at 96-hours. Total alkalinity, hardness (EDTA), and conductivity were measured at the beginning of the test in the water control.

Remark : Reliability: Medium because a suboptimal study design with nominal concentrations was used for testing.

Result : No mortality was observed during the test; therefore, an LC50 value could not be determined, but is > 10,000 mg/L.

The chemical and physical parameters measured during the test were all within acceptable limits. Dissolved oxygen ranged from 7.6-8.8, 7.8-8.8, 7.3-8.8, 7.1-8.8 in the 0, 1, 1000, and 10,000 mg/L groups, respectively.

pH ranged from 7.0-7.5, 6.9-7.5, 6.8-7.4, and 6.5-6.9 for the 0, 1, 1000, and 10,000 mg/L groups, respectively.

Total alkalinity, EDTA hardness, and conductivity at 0 hours in the control group were 83 mg/L as CaCO3, 76 mg/L as CaCO3, and 179 µmhos/cm, respectively.

Test substance : N-methylformamide, purity 97.8 mole %

02.02.2006 (45)

Type	:	static
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	> 5000
Method	:	other
Year	:	1993

GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. Nominal concentrations of 0, 0.50, 1.0, 50, 500, and 5000 mg/L were used for testing. The test was unaerated and static. Dissolved oxygen and pH were measured.
Remark	:	Reliability: Medium because a suboptimal study design with nominal concentrations was used for testing.
Result	:	Based on visual observation, the test substance was soluble in well water at all concentrations. No mortality was observed in any test concentration. All fish in the highest test concentration (5000 mg/L) were noted to be breathing rapidly after 24 hours of exposure and for the duration of the test. Loading (0.15 g/L at test end) was within acceptable limits. Dissolved oxygen concentrations were 8.3, 8.3, 8.3, 8.3, 8.3, 8.3 mg/L at 0 hours and 4.5, 5.6, 5.4, 5.5, 5.1, and 2.2 mg/L at 96 hours in the 0, 0.50, 1.0, 50, 500, and 5000 mg/L groups, respectively. pH values were 7.6, 7.6, 7.6, 7.6, 7.5, and 7.2 at 0 hours and 7.3, 7.3, 7.3, 7.2, 7.2, and 6.8 at 96 hours in the 0, 0.50, 1.0, 50, 500, and 5000 mg/L groups, respectively.
Test substance	:	N-methylformamide, purity 99.9%

06.02.2006 (48)

Type	:	other
Species	:	other: Fish
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	39170
Method	:	other: Modeled
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Reliability: Estimated value based on accepted model.
Result	:	96-hour LC50 (fish) = 39,170 mg/L (log10 Kow of -1.14)

02.02.2006 (126)

Remark	:	Data from this additional source supports the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

02.02.2006 (12)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC0	:	500
EC50	:	> 500
EC100	:	> 500
24-hour EC50	:	> 500
Method	:	other

Year	:	1989
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	The procedures used in the test were based on the recommendations of the following guideline: EEC guideline 79/831/ECG, appendix V, part C. The test containers were 20 mL flat-bottom test tubes. The test volume was 10 mL. There were 5 test animals per test tube with a total of 20 test animals per concentration (4 test tubes per concentration). The nominal concentration of the stock solution was 500 mg/L. The stock solution was diluted with water to obtain the test concentrations of 0, 62.5, 125, 250, and 500 mg/L. The ability to swim was used in this test as a substitute criterion for liveability. Animals were considered unable to swim if, after knocking on the test vessels, no swimming movements occurred within 15 seconds. Swimming ability was assessed at 0, 3, 6, 24, and 48 hours. Temperature measurements were recorded after 0, 24, and 48 hours in a separte vessel next to the test vessels. pH and O2 measurements were recorded after 0 and 48 hours in 1 test duplicate per concentration.
Remark	:	Reliability: Medium because a suboptimal study design with nominal concentrations was used for testing.
Result	:	24-48 hour EC0 = 500 mg/L 24-48 hour EC50 > 500 mg/L 24-48 hour EC100 > 500 mg/L The test temperature was reported as 21.5°C. The pH was 8.1, 8.3, 8.2, 8.2, and 8.1 at 0 hours in the 0, 62.5, 125, 250, and 500 mg/L groups, respectively. The pH was 8.0, 8.1, 8.1, 8.1, and 8.0 at 48 hours in the 0, 62.5, 125, 250, and 500 mg/L groups, respectively. The oxygen content was 9.1, 9.2, 9.0, 9.0, and 9.1 mg/L at 0 hours in the 0, 62.5, 125, 250, and 500 mg/L groups, respectively. The oxygen content was 8.4, 8.5, 8.5, 8.4, and 8.4 mg/L at 48 hours in the 0, 62.5, 125, 250, and 500 mg/L groups, respectively. Conductivity was measured as 650 µS/cm and total hardness was measured as 2.68 mmol/L in the test water.
Test substance	:	N-methylformamide, purity 99.5%
06.02.2006		(14)

Type	:	other
Species	:	Daphnia sp. (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	33787
Method	:	other: Modeled
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Reliability: Estimated value based on accepted model.
Result	:	48-hour EC50 (daphnids) = 33,787 mg/L (log10 Kow of -1.14)
02.02.2006		(126)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Scenedesmus subspicatus (Algae)

Endpoint	:	other: Biomass and growth rate
Exposure period	:	72 hour(s)
Unit	:	mg/l
Method	:	other
Year	:	1992
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4

Method : The procedures used in the test were based on the recommendations of the following guideline: DIN 38 412/9.

The concentrations tested were 0, 5.0, 8.0, 12.8, 20.5, 32.8, and 42.4 g/L. Four duplicate experiments were run for each concentration and control. Room temperature was maintained at 20±1°C. The experiment was conducted under continuous illumination. The cell concentrations were determined after 0, 24, 48, and 72 hours using a fluorimeter. The pH value was measured at the beginning and the end of the study in the control, and at all concentrations. The algae were kept in suspension by shaking twice daily on a test tube shaker. The stock solutions were prepared in a graduated cylinder at a concentration of 104.8 g/L with sterile twice distilled water. No solvent was used.

The computer evaluation of the inhibition was carried out using the probit method. The rate-related inhibition could not be evaluated by computer using the probit method. After 96 hours, the algal cell count was no longer doubled, and the experiment was ended.

The initial concentration of algal cells was 10,000 cells/mL. The experiment was conducted with continuous illumination (10,000 lux, white light or 0.72x10E20 photons m2s or 120µE/m2s.

Remark : Reliability: Medium because a suboptimal study design with nominal concentrations was used for testing.

Result : Growth inhibition results (0-72 hours):

HB 10 (72 hours): 13.88 g/L
HB 50 (72 hours): 17.30 g/L
HB 90 (72 hours): 21.57 g/L

Rate-related inhibition (0-72 hours):

Hµ 10 (72 hours): ---
Hµ 50 (72 hours): ---
Hµ 90 (72 hours): ---

Concentration of lowest inhibition (83.75%): 20.50 g/L
Concentration of highest inhibition (100%): 32.80 g/L
Growth promotion: 12.8 g/L

The fluorescence measurement in the control increased by a factor of 20x indicating an acceptable control performance.

The pH was 8.2, 8.2, 8.1, 8.1, 8.1, 8.1, 8.1 at 0 hours for the 0, 5.0, 8.0, 12.8, 20.5, 32.8, and 52.4 g/L groups, respectively. The pH was 8.7, 8.7, 8.6, 8.8, 8.6, 8.3, 6.1 at 72 hours for the 0, 5.0, 8.0, 12.8, 20.5, 32.8, and 52.4 g/L groups, respectively.

Additional results are shown in the tables below.

Biomass

Concentration g/L	Biomass	Inhibition in %

0	1.11	-15.01
5.0	1.27	-6.88
8.0	1.18	-2.26
12.8	1.13	83.75
20.5	0.18	100
32.8	---	100
52.4	---	---

Growth Rate

Concentration g/L	Growth Rate	Rate-related Inhibition in %
0	1.01	-3.62
5.0	1.04	-1.66
8.0	1.02	-3.54
12.8	1.04	55.23
20.5	0.45	137.97
32.8	-0.38	---
52.4	---	---

Test substance : N-methylformamide, purity not reported
02.02.2006 (15)

Species : other algae: Green algae
Endpoint : other
Exposure period : 96 hour(s)
Unit : mg/l
EC50 : 17630
Method : other: Modeled
Year :
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : Reliability: Estimated value based on accepted model.
Result : 96-hour EC50 (green algae) = 17,630 mg/L (log10 Kow of -1.14)
02.02.2006 (126)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	4000 mg/kg bw
Species	:	rat
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	other
Year	:	1962
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Reliability: Not assignable because limited study information was available.
Test substance	:	N-methylformamide, purity not reported

02.02.2006 (178)

Type	:	LD50
Value	:	7077 mg/kg bw
Species	:	rat
Strain	:	Long-Evans
Sex	:	female
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	other
Year	:	1971
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Reliability: Not assignable because limited study information was available.
Test substance	:	N-methylformamide, purity not reported

02.02.2006 (179)

Type	:	LD50
Value	:	2600 mg/kg bw
Species	:	mouse
Strain	:	Balb/c
Sex	:	female
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	other
Year	:	1985
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

At least 5 dose levels were employed. Mortality was assessed on day 30 post-administration. Lethal dose values and their 95% confidence limits were computed according to the method of Litchfield, J. T. and F. Wilcoxon

(1949). J. Pharmacol. Exp. Ther., 96:99.

Remark : Reliability: Medium because a scientifically defensible study design was used, but limited study data were available.

Result : LD50 = 2600 mg/kg (95% confidence limits 2031-3328 mg/kg)

LD10 = 1800 mg/kg (95% confidence limits 1200-2700 mg/kg)

Test substance : N-methylformamide, purity not reported

02.02.2006 (101)

Type : LD50
Value : 2650 mg/kg bw
Species : mouse
Strain : other: Dub/ICR
Sex :
Number of animals :
Vehicle :
Doses :
Method : other
Year : 1979
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : Reliability: Not assignable because limited study information was available.
Result : Time of death was between 1 - 8 days.
Test substance : N-methylformamide, purity not reported

07.02.2006 (191)

Remark : Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

03.02.2006 (100)

5.1.2 ACUTE INHALATION TOXICITY

Type : other: ALC
Value : > 10.76 mg/l
Species : rat
Strain : other: Crl:CD®
Sex : male
Number of animals :
Vehicle :
Doses : 0.69, 1.0, 5.6, and 10.76 mg/L
Exposure time : 4 hour(s)
Method : other
Year : 1982
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Method : No specific test guideline was reported. Rats were exposed to the test substance in a single 4-hour exposure. Body weights and clinical signs were assessed. Six rats were exposed in each rangefinder group. Exposure concentrations were 0.69, 1.0, 5.6, and 10.76 mg/L.

Remark : Reliability: Medium because a suboptimal study design was used.

Result : No deaths occurred during the rangefinding study. Rats exposed to 5.6 and 10.76 mg/L showed severe weight loss followed by weight gain. Gasping was observed during exposure in 2/6 rats in the 10.76 mg/L group.

Test substance : N-methylformamide, purity 99.5%

02.02.2006 (43)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	other: ALD
Value	:	
Species	:	other: Pregnant ChR-CD and Sprague-Dawley rats and pregnant New Zealand White rabbits
Strain	:	
Sex	:	female
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	other
Year	:	1966
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. Rats weighed 220-250 grams and rabbits weighed 4 kg at study start. MMF was applied with a syringe to the back skin (thoracic vertebral area) after the hair had been clipped. In determining the ALD, one animal was used per dose level with a factor of 1.5 between dose levels. Rats were treated on day 11 and rabbits were treated on day 15 of gestation. Surviving rats were sacrificed on day 21 and surviving rabbits on day 30 of gestation.
Remark	:	Reliability: Medium because a suboptimal study design was used. Pregnant animals were used in the study and limited study information was available.
Result	:	ALD (Rat) = 11,000 mg/kg ALD (Rabbit) = 1500 mg/kg
Test substance	:	N-methylformamide, commercial grade with <2% impurities

06.02.2006 (37) (176)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

5.2.2 EYE IRRITATION

Species	:	rabbit
Concentration	:	
Dose	:	
Exposure time	:	
Comment	:	
Number of animals	:	
Vehicle	:	
Result	:	
Classification	:	
Method	:	OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year	:	1992
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4

Method : The procedures used in the test were based on the recommendations of OECD Guideline 405: Acute Eye Irritation/Corrosion (May 12, 1981).

MMF (100 µL) was applied to the lower conjunctival sac of six rabbits. The Draize scoring criteria was used when eyes were evaluated at 4, 24, 48, 72, and 96 hours post-instillation. One drop of 2% sodium fluorescein was applied to the eyes before visual scoring of percentage corneal damage.

Remark : Reliability: High because a scientifically defensible or guideline method was used.

Result : MMF was given an EEC classification of "Irritating to eyes" due to mean scores over 24/48/72 hours for conjunctivitis greater than two.

Mean score of Conjunctivitis (max of 3) [Time after application: 4, 24, 48, 72, and 96 hours, respectively]: 2.3, 2.4, 2.2, 2.0, and 1.5

Mean score of chemosis (max of 4) [Time after application: 4, 24, 48, 72, and 96 hours, respectively]: 2.0, 1.2, 1.0, 0.3, and 0.0

Mean score of iritis (max of 2) [Time after application: 4, 24, 48, 72, and 96 hours, respectively]: 1.0, 0.7, 0.0, 0.0, and 0.0

Mean score of corneal opacity (max of 4) [Time after application: 4, 24, 48, 72, and 96 hours, respectively]: 1.0, 0.8, 0.7, 0.3, and 0.0

Mean surface of corneal damage (100% max) [Time after application: 4, 24, 48, 72, and 96 hours, respectively]: 43, 60, 32, 11, and 10

Test substance : N-methylformamide, purity not reported

02.02.2006 (83)

Remark : Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

02.02.2006 (27)

5.3 SENSITIZATION

5.4 REPEATED DOSE TOXICITY

Type	:	
Species	:	rat
Sex	:	male
Strain	:	other: Crl:CD®
Route of admin.	:	inhalation
Exposure period	:	2 weeks
Frequency of treatm.	:	6 hours/day, 5 days/week
Post exposure period	:	14 days
Doses	:	0, 0.1, 0.3, 1.0 mg/L (0, 50, 132, 402 ppm)
Control group	:	yes
NOAEL	:	.12 mg/l
Method	:	other
Year	:	1983
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Method : No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

Rats were 7-8 weeks old and weighed 207-242 grams at study start.

Inhalation exposures were nose-only. The liquid test material was syringe-driven into a heated (180°C) 3-neck flask, where it was flash evaporated. Unheated dilution air passed through the flask and carried vapors to the exposure chamber. Atmospheric concentrations were periodically measured and analyzed via gas chromatography. Chamber temperature was monitored with a thermometer during each exposure.

All rats were weighed and observed daily (excluding weekends) throughout the exposure and recovery periods.

Clinical laboratory measurements were made on urine samples collected overnight following the 9th exposure and the 13th day of recovery. Blood samples were taken from the rats' tails after the 10th exposure and the 14th day of recovery. Approximately 13 hematological parameters were measured or calculated, 7 clinical chemistry parameters were measured or calculated, and 12 urine chemistry parameters were measured or examined.

After the 10th exposure, 5 rats from each group were selected at random and sacrificed for gross and histopathological examination. Five of the remaining rats were sacrificed on the 14th day of recovery for identical examination. Approximately 25 organs and/or tissues were saved for microscopic examinations. Seven organ weights (heart, liver, lungs, kidneys, spleen, testes, and thymus) were recorded. Organs and tissues examined included adrenal glands, thyroid gland, esophagus, stomach, duodenum, pancreas, jejunum, ileum, cecum, colon, liver, spleen, thymus, mediastinal lymph nodes, eye, brain, trachea, heart, nose, urinary bladder, lungs, sternum, kidneys, testes, and epidiymides.

Of the 15 rats/group exposed to the test material, 5/group were used solely for collection of urine samples for MMF analysis. Urine samples were collected overnight from these rats on exposure days 1, 4, and 9 and on recovery days 3, 6, and 13. Samples were analyzed by gas chromatography.

The body weight, hematologic, and clinical chemistry data were statistically analyzed by a one-way analysis of variance. Least significant differences and Dunnett tests were used to compare MMF-treated rats with the controls when the ratio of variance indicated a significant, among to within group variation.

Remark : Reliability: High because a scientifically defensible or guideline method was used.

Result : Mean measured exposure concentrations were 0.12, 0.32, and 0.97 mg/L for the 0.1, 0.3, and 1.0 mg/L groups, respectively. Chamber temperature was maintained at 27-34°C.

Clinical observations of rats exposed to 0.12 mg/L were indistinguishable from controls throughout the study. Rats exposed to 0.32 mg/L had significantly lower body weights during the first week and the latter part of the second week of exposure. Gain during the recovery phase was parallel to that of controls. Rats exposed to 0.97 mg/L had significantly lower body weights throughout the study with severe weight depression during the exposure phase and weight gain at a rate parallel to that of controls during the recovery phase.

Clinical chemistry measurements made at the end of the exposure period showed no compound-related effects in rats exposed to 0.12 mg/L. Rats exposed to 0.32 and 0.97 mg/L had increased serum cholesterol concentrations. Rats exposed to 0.97 mg/L also had decreased serum urea nitrogen concentrations, decreased serum alkaline phosphatase

activities, and increased serum ALT/GPT and AST/GOT activities. These changes were interpreted to be evidence of treatment-related effects on the integrity and function of hepatic tissue. All compound-related effects observed at the end of the exposure period were absent 14 days later.

Pathologic examination revealed no compound-related macroscopic lesions in any rats. Microscopically, there were compound-related effects following exposure in the livers of rats exposed to 0.32 and 0.97 mg/L. Lesions included pale cytoplasm, increase in the number of mitotic figures, and cytoplasmic lipid vacuolation. These changes were interpreted as being degenerative and regenerative in nature. Fourteen days following exposure, partial recovery at the high level and complete recovery at the intermediate level had occurred. Incidence levels of the microscopic findings mentioned above can be found in the following tables.

Pale cytoplasm [Exposure Concentrations 0, 0.12, 0.32, and 0.97 mg/L, respectively]: 0/5(a);0/5(b), 0/5(a);0/5(b), 5/5(a);1/5(b), and 5/5(a);5/5(b)

Increase in mitotic figures [Exposure Concentrations 0, 0.12, 0.32, and 0.97 mg/L, respectively]: 0/5(a);0/5(b), 0/5(a);0/5(b), 3/5(a);0/5(b), and 2/5(a);0/5(b)

Cytoplasmic lipid vacuolation [Exposure Concentrations 0, 0.12, 0.32, and 0.97 mg/L, respectively]: 0/5(a);0/5(b), 0/5(a);0/5(b), 1/5(a);0/5(b), and 5/5(a);0/5(b)

a = incidence following exposure period
b = incidence following recovery period

A comparison of organ weights between test rats and controls showed no changes in rats exposed to 0.12 mg/L. Following 10 exposures, mean absolute weights of the heart, lung, spleen, and thymus were significantly lower in rats exposed to 0.97 mg/L than in controls. On a relative basis, rats exposed to 0.32 and 0.97 mg/L had increased liver/body weight ratios. Fourteen days following exposure, there were no differences between test rats and controls. Although relative testes weights were increased at 0.97 mg/L following 10 exposures, no microscopic findings were observed in the testes, and no differences in testes weight were found fourteen days following exposure.

MMF was excreted in a dose-dependent fashion in the urine of exposed rats. It was detected in the urine of rats exposed to 0.32 and 0.97 mg/L on day 1 and in rats exposed to 0.12 mg/L on day 4. In all test groups, urinary levels of MMF generally increased throughout the exposure period and then decreased throughout the recovery period. At the end of the recovery period, MMF was still detectable in rats exposed to 0.32 and 0.97 mg/L, but not in rats exposed to 0.12 mg/L.

The no-effect level of the study was determined to be 0.12 mg/L. The intermediate and high levels exhibited dose dependent effects with primary effects on the liver.

Test substance : N-methylformamide, purity 99.5%

14.02.2006 (44) (91)

Remark : Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

02.02.2006 (24) (40)

Remark : Supporting Data for Dimethylformamide (DMF)

06.02.2006

Type	:	
Species	:	rat
Sex	:	
Strain	:	
Route of admin.	:	inhalation
Exposure period	:	10 days
Frequency of treatm.	:	0.5 or 6 hours/day
Post exposure period	:	no
Doses	:	0, 91, 1104, or 91 and 841 ppm
Control group	:	yes
Method	:	other
Year	:	1963
GLP	:	no
Test substance	:	other TS
Method	:	No specific test guideline was reported. Four test groups were employed in this preliminary study. Group I received 91 ppm, 6 hours/day for 10 days. Group II received 1104 ppm, 0.5 hours/day for 10 days. Group III received 91 ppm, 6 hours/day for 10 days followed by a 841 ppm exposure for 0.5 hours at the end of the 10th exposure. The CT (concentration, ppm x time, hours) for the groups were 5460, 5520, and 5880 ppm x hours for Groups I, II , and III, respectively. The control group received air for 6 hours/day for 10 days.
Remark	:	Reliability: Medium because a sub-optimal study design was used. Study was only a preliminary study and complete study details were not reported.
Result	:	Liver weights of the test rats were elevated and the liver to body weight ratios of the test groups were statistically greater than the controls. Liver to body weight ratio (%) [Exposure Group I, II, III, and Control, respectively]: 4.39, 4.36, 4.57, and 4.02 Liver weight range (g) [Exposure Group I, II, III, and Control, respectively]: 13.3-18.9, 13.0-17.6, 13.7-18.7, and 12.0-17.1
Test substance	:	DMF, purity not reported

06.02.2006 (25)

Type	:	
Species	:	other: Fisher 344 rats and B6C3F1 mice
Sex	:	male/female
Strain	:	
Route of admin.	:	inhalation
Exposure period	:	90 days
Frequency of treatm.	:	5 days/week, 6 hours/day
Post exposure period	:	no
Doses	:	0, 50, 100, 200, 400, and 800 ppm
Control group	:	yes
Method	:	other
Year	:	1992
GLP	:	yes
Test substance	:	other TS
Method	:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. Rats (30 per sex per group) and mice (10 per sex per group) were exposed via whole-body inhalation. A counter-current distillation system was used to generate vapors from liquid-state DMF. A fresh aliquot of DMF was transported daily, from the glass reservoir using a metering pump and Teflon lines, to the counter-current distillation column. Conditioned room

air supplied primary dilution air for the distillation column. The test vapor was generated at the highest exposure concentration (800 ppm), delivered to the chamber through a common distribution manifold, and then diluted to target concentrations at each exposure chamber. Analytical concentrations were determined via infrared analysis.

Rats were 51 days of age at the first exposure. Mice were 46 days of age at first exposure. For the rats, each study group was divided into three subgroups of 10 rats per sex each. The three subgroups consisted of a base study group, a cardiovascular group, and a renal function group.

Animals were observed twice daily for mortality and moribundity. Body weights were measured weekly throughout the study and at necropsy.

Sperm morphology and vaginal cytology evaluations were performed on rats and mice exposed to 0, 50, 200, and 800 ppm DMF. Sperm morphology was evaluated at necropsy. Vaginal cytology was done by vaginal lavage with saline during the 2 weeks prior to necropsy.

Clinical pathology evaluations were conducted on cardiovascular study rats at 4 and 23 days and on base-study rats at 13 weeks. Urinalysis was performed on 5 rats/sex in the 0, 50, 200, and 800 ppm groups. Kidney histology was performed on these animals.

Blood pressure and electrocardiograms were measured within 24 hours of the last DMF exposure in the cardiovascular group rats. The animals were killed and the heart removed for microscopic evaluation.

At study termination, rats in the base study and the renal function groups and mice were killed and complete necropsies were performed. Examination for gross lesions was conducted and liver, thymus, kidneys, testicles, heart, and lung weights were recorded. The liver was microscopically examined in all dose groups. In addition, approximately 35 other organs or tissues were examined histologically in the control and high-dose groups.

Organ, body weight, blood pressure, and electrocardiographic data were analyzed using the parametric comparisons procedures of Williams, D. A. (1971). Biometrics, 27:103-117, Williams, D. A. (1972). Biometrics, 28:519-531, and Dunnett, W. (1955). J. Amer. Stat. Assoc., 50:1095-1121. Clinical chemistry and hematology data were analyzed using nonparametric multiple comparisons methods of Shirley, E. (1977). Biometrics, 33:386-389 and Dunn, O. J. (1964). Technometrics, 6:241-252. Jonckheere's test was used to assess significance of dose-response trends, and to determine whether a trend-sensitive test (Williams, Shirley) was more appropriate for pairwise comparisons than a test capable of detecting departures from monotonic dose response (Dunnett, Dunn). The outlier test of Dixon, W. and F. Massey (1951). Introduction to Statistical Analysis, pp. 145-147, McGraw Hill, NY was used to detect extreme values.

Remark : Reliability: High because a scientifically defensible or guideline method was used.

Result : The analytical concentrations were 0, 50.2, 98.6, 198.1, 401.3, and 804.6 ppm for the 0, 50, 100, 200, 400, and 800 ppm groups, respectively.

Rats

There was no substance-related mortality. Body weight gains were reduced by approximately 47-65% in rats exposed to 800 ppm and to a lesser degree in the animals of the 400 ppm group.

Evidence for hepatocellular injury was seen as early as day 4 as increases in activities of liver-specific enzymes (e.g. ALT, SDH, and ICDH) in the

serum of both sexes at 200-800 ppm DMF. Serum cholesterol levels were increased in all exposed rats at all time points (4, 24, and 91 days).

ALT (Alanine aminotransferase in IU/L) - Day 4
[0, 50, 100, 200, 400, and 800 ppm male and female groups, respectively]:
47, 42; 45, 41; 49, 40; 53*, 41; 74**, 46; and 356**, 172**

SDH (sorbitol dehyrogenase in IU/L) - Day 4
[0, 50, 100, 200, 400, and 800 ppm male and female groups, respectively]:
20, 23; 19, 24; 23, 23; 28**, 28**; 43**, 40**; and 130**, 103**

ICDH (isocitrate dehydrogenase in IN/L) - Day 4
[0, 50, 100, 200, 400, and 800 ppm male and female groups, respectively]:
15.01, 11.90; 11.53, 12.74; 12.18, 12.17;
12.74, 15.36; 14.57, 13.51; and 32.91*, 30.21**

Serum cholesterol (in mg/dL) - Day 4
[0, 50, 100, 200, 400, and 800 ppm male and female groups, respectively]:
75, 97; 97**, 120**; 112**, 137**; 112**, 152**; 116**, 141**; and 109**, 138**

Serum cholesterol (in mg/dL) - Day 24
[0, 50, 100, 200, 400, and 800 ppm male and female groups, respectively]:
70, 89; 81**, 106**; 82**, 106**; 84**, 117**; 81**, 111**; and 91**, 117**

Serum cholesterol (in mg/dL) - Day 91
[0, 50, 100, 200, 400, and 800 ppm male and female groups, respectively]:
83, 97; 94*, 109**; 102**, 129**; 98**, 115**; 98**, 137**; and 134**, 136**

* - Significantly different (P equal to or less than 0.05) from control group by Dunn's or Shirley's test.
** - Significantly different (P equal to or less than 0.01) from control group by Dunn's or Shirley's test.

Relative liver weights were increased in the males at 100 ppm and above and at all concentrations of the females. Minimal to moderate centrilobular hepatocellular necrosis was seen in both sexes at 400 and 800 ppm and pigment accumulation (hemosiderin and lipfuscin) in macrophages and Kupffer cells was found in both sexes at 800 ppm.

Relative liver weights (in mg organ weight/g body weight)
[0, 50, 100, 200, 400, and 800 ppm male and female groups, respectively]:
38.03, 33.91; 40.50, 37.19**; 44.33**, 39.45**; 46.33**, 38.31**; 45.31**, 40.39**; and 40.15**, 36.81**

* - Significantly different (P equal to or less than 0.05) from control group by Williams' or Dunnett's test.
** - Significantly different (P equal to or less than 0.01) from control group by Williams' or Dunnett's test.

Hepatocyte necrosis [0, 50, 100, 200, 400, and 800 ppm male and female groups, respectively]: 0/10, 0/10; 0/10, 0/10; 0/10, 0/10; 0/10, 0/10; 10/10, 8/10; and 10/10, 10/10

Macrophage pigment [0, 50, 100, 200, 400, and 800 ppm male and female groups, respectively]: 0/10, 0/10; 0/10, 0/10; 0/10, 0/10; 0/10, 0/10; 0/10, 0/10; and 10/10, 10/10

Prolonged diestrus was observed in 7 of 10 females exposed at 800 ppm (i.e. at a concentration that produced hepatotoxicity and reduced body weight gain).

Relative testis weights were increased at 400 and 800 ppm DMF; however, no microscopic findings or adverse effects on sperm density or motility were observed.

Mice

Five male mice died of undetermined causes during the study; 3 of these were in the lowest exposure group, suggesting that exposure to DMF was not involved. All female mice survived the 13-week exposure period. No exposure-related clinical signs were observed. Reduced body weight gain was noted in the 800 ppm female mice.

Relative and/or absolute kidney and lung weights were variably increased in all exposed groups of females. Both absolute and relative thymus weights were decreased in the 50 ppm male mice. This finding was not considered biologically significant. Absolute liver weights were moderately increased in the 200-800 ppm males and 50-800 ppm females. Relative liver weights were increased in both sexes at all exposure levels.

Absolute liver weights (in grams) [0, 50, 100, 200, 400, and 800 ppm male and female groups, respectively]: 1.668, 1.171; 1.907, 1.306*; 1.574, 1.477**; 2.074**, 1.756**; 2.020**, 1.699**; and 1.940**, 1.514**

Relative liver weights (in mg organ weight/g body weight)
[0, 50, 100, 200, 400, and 800 ppm male and female groups, respectively]: 49.13, 46.41; 56.94*, 49.73*; 51.26*, 54.23**; 60.53**, 61.44**; 60.74**, 62.92**; and 62.40**, 61.55**

* - Significantly different (P equal to or less than 0.05) from control group by Williams' or Dunnett's test.
** - Significantly different (P equal to or less than 0.01) from control group by Williams' or Dunnett's test.

No significant changes in the reproductive system evaluations were observed in male mice. In females, there was a significant trend toward an increase in the estrous cycle length. The animals in the 200 ppm group spent significantly more time in the stages of estrus and diestrus than did the controls.

Estrous cycle length (in days) [0, 50, 200, and 800 ppm females, respectively]: 4.15; 4.05; 4.55; and 4.80

Centrilobular hepatocellular hypertrophy (minimal to mild) was found in all groups of male mice and in female mice exposed to 100 ppm or more.

Liver lesions [0, 50, 100, 200, 400, and 800 ppm male and female groups, respectively]: 0/10, 0/10; 4/10, 0/10; 9/10, 10/10; 10/10, 10/10; 10/10, 10/10; and 10/10, 10/10

Test substance : DMF, purity > 99%
07.02.2006 (143)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Escherichia coli reverse mutation assay
System of testing : Escherichia coli, strains Sd-4, Sd-4-73, WP-14, WP-2
Test concentration : 10, 20, 50 mg/mL
Cycotoxic concentr. :
Metabolic activation : without
Result : negative
Method : other

Year	:	1955
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	No specific test guideline was reported. The test material was dissolved in sterile distilled water. The bacteria were grown for 24 hours at 37°C in an aerated broth culture. The streptomycin-dependent strains were supplemented with 10 µg of streptomycin per mL culture media. Five mL samples of fully grown culture were centrifuged, washed in saline, and resuspended in distilled water or buffer. These suspensions were kept for 1 to 3 hours at 37°C. After treatment, suspensions showing microscopically detectable clumping were not used in the experiment. The numbers of viable bacteria in the control and treated suspensions were assayed by plating samples of suitable dilutions on nutrient broth agar, supplemented with streptomycin if applicable. In the auxotrophic strains, the number of revertants to nondeficiency was determined by plating samples of the suspension on synthetic medium enriched with dehydrated nutrient broth. The streptomycin-dependent strains were plated on nutrient broth agar in order to determine the number of reversions to nondependence. Reversions of cys-2 in strain Sd-4-73 were scored by plating on enriched synthetic medium to which streptomycin had been added. The frequency of observed mutations was obtained by dividing the number of mutant colonies, corrected for the estimated number of spontaneously occurring mutants, by the number of viable bacteria plated, and recalculating on the basis of 10E8 viable cells.
Remark	:	Reliability: Low because an inappropriate method or study design was used.
Test substance	:	N-methylformamide, purity not reported
03.02.2006		(67)
Remark	:	Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.
03.02.2006		(4) (100)
Remark	:	Supporting data for dimethylformamide (DMF).
03.02.2006		
Type	:	Bacterial reverse mutation assay
System of testing	:	Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100
Test concentration	:	0, 0.94x10E4, 2.4x10E4, 4.7x10E4, 9.4x10E4, 19x10E4, 47x10E4 µg/plate
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	negative
Method	:	other
Year	:	1977
GLP	:	no
Test substance	:	other TS
Method	:	No specific test guideline was reported; however, procedures generally followed those outlined in Ames et al. (1975). Mutat. Res., 31:347-364. In the absence of an activation system, a solution of the test sample and approximately 10E8 bacteria were added to top agar. These components

were mixed and poured on the surface of a plate containing Davis minimal agar. To treat in the presence of an activation system, S9 mix was added to the bacteria-test sample-top agar mixture. The S9 mix contained S9, MgCl2, KCl, glucose-6-phospahte, NADP, and sodium phosphate. Once the S9 was added to the test sample and top agar, the components were mixed and immediately poured over the minimal agar plate.

The revertant colonies were counted after the plates were incubated at 37°C for 48 hours.

Duplicate plates (1 experiment) were used in the test condition which included metabolic activation and duplicate plates (2 experiments) were used in the test condition without metabolic activation.

Positive controls (2-aminoanthracene (2-AA), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), 9-aminoacridine (9AAc), and 2-nitrofluorene (2NF)) and negative controls (solvent) were included in each assay.

The cytotoxicity of the test sample in the presence and absence of an activation system, as measured in strain TA1535, was the basis for selecting concentrations to be used in the mutagenesis experiment. The protocol used to determine cytotoxicity was identical to the mutagenesis protocol except that 10E3 rather than 10E8 bacteria were used per plate and a non-limiting concentration of histidine was present.

Data from replicate plates within a single experiment were averaged. The average of these values from different experiments was determined. The highest average number of revertants that was obtained was expressed as a multiple of the control value for the sensitive strain(s). When a test sample was active, the average numbers of revertants observed before activity plateaus or decreases at the various concentrations tested were submitted to linear regression analysis. The slope of the line thus obtained was used to determine the number of revertants/nmole or µg of test sample.

A chemical was classified as non-mutagenic if the reversion frequency was less than 2 times the spontaneous frequency, and if less than 0.02 revertants/nmole are observed.

Remark : Reliability: High because a scientifically defensible or guideline method was used.

Result : The cytotoxicity data for DMF is provided in the table below.

S9	Concentration (µg/plate)	% of Control Survival
-	47x10E4	<0.2
-	19x10E4	70
+	47x10E4	20
+	19x10E4	50

Normally, concentrations of test sample that give less than 50% of control survival are not selected for the mutagenesis assay. However, since these experiments were initiated to aid in evaluating a Utah Biological Testing Service (UBTS) Report, 500 µg/plate was chosen as the highest concentration.

A previous study conducted by DuPont (DuPont Co. (1976). Unpublished data, Haskell Laboratory Report No. HL 424-76) indicated that DMF tested at concentrations equal to or less than 10 mg/plate were not mutagenic in the Ames test. However, the UBTS reported that DMF was mutagenic (tested at concentrations greater than 10 mg/plate). This data prompted DuPont to conduct the present test. Data from this test indicated that DMF

was not mutagenic, even at concentrations that are cytotoxic. However, by testing a toxic concentration of DMF, a possible technical explanation for the conclusions of the UBTS study became apparent. While the number of revertants per plate at all concentrations of DMF never rose above the spontaneous frequency, a substantial number (about 1000) of small colonies (mutants, not revertants) was present in dishes that received 500 µL of DMF per plate. This effect was observed in all strains. The dishes that contained these small colonies did not possess the background lawn of mutants that are present under conditions of high survival. The presence or absence of a background lawn was confirmed by microscopic observation. The absence of a background lawn is indicative of toxicity.

Colonies from TA98 and TA1535 that received 500 µL and 100 µL of DMF were selected and streaked out on fresh plates that did or did not contain histidine. The colonies from the 100 µg plates were counted as real revertants. A survivor of toxicity (a mutant) should be able to grow in the presence of histidine, but not its absence, while a revertant should grow under both conditions. None of the colonies from the 500 µL plate grew in the absence of histidine while all grew in its presence. Since biotin was not present in the -his plates and the colonies plated under the 2 conditions were not identical, each colony that grew on the complete plate was restreaked on -his, +his, and complete plates. The results confirmed that the colonies were composed of mutants, not revertants. Colonies (5/5) that were considered to be revertants from TA98 and TA1535 that received 100 µL of DMF grew on -his plates.

These data clearly show that the colonies that appeared when highly toxic concentrations were used, were survivors from the parent mutant population, not revertants to histidine in dependence. It appears that these surviving mutants were counted as revertants in the UBTS study, leading to their conclusion that DMF was mutagenic.

The expected positive results were observed in the cells treated with the positive controls.

Mutagenic activity (# of revertants) are detailed below.

With Metabolic Activation [TA1535, TA1537, TA1538, TA98, and TA100, respectively]:

Control, 20, 9, 31, 48, and 166;
0.94x10E4 ug/plate of DMF, 16, 11, 28, 45, and 148;
2.4x10E4 ug/plate of DMF, 16, 16, 24, 35, and 151;
4.7x10E4 ug/plate of DMF, 14, 10, 25, 42, and 127;
9.4x10E4 ug/plate of DMF, 15, 5, 25, 25, and 121;
19x10E4 ug/plate of DMF, 12, 8, 16, 21, and 73;
47x10E4 ug/plate of DMF, T, T, T, T, and 20

Positive Control With Metabolic Activation [TA1535, TA1537, TA1538, TA98, and TA100, respectively]:

2AA (5 ug/plate): NT, NT, NT, NT, and 2146
2AA (10 ug/plate): 322, NT, 2225, 2897, and NT
2AA (100 ug/plate): NT, 320, NT, NT, and NT

T = toxicity as indicated by sparse background lawn
NT = Not tested

Without Metabolic Activation [TA1535, TA1537, TA1538, TA98, and TA100, respectively]:

Control, 18, 8, 15, 25, and 142;

0.94x10E4 ug/plate of DMF, 18, 8, 9, 18, and 143;
2.4x10E4 ug/plate of DMF, 13, 10, 14, 20, and 148;
4.7x10E4 ug/plate of DMF, 15, 8, 9, 15, and 142;
9.4x10E4 ug/plate of DMF, 14, 6, 12, 12, and 122;
19x10E4 ug/plate of DMF, 7, 5, 3, 10, and 69;
47x10E4 ug/plate of DMF, 2, T, T, T, and 17

Positive Controls Without Metabolic Activation [TA1535, TA1537, TA1538, TA98, and TA100, respectively]:

MNNG (2 µg/plate): 2710, NT, NT, NT, and 2695
9AAc (50 µg/plate): NT, 1544, NT, NT, NT
2NF (25 µg/plate): NT, NT, 2275, 2916, and NT

T = toxicity as indicated by sparse background lawn
NT = Not tested

Test substance : DMF, purity 100%

15.02.2006 (42)

Remark : Data from similar assays reported in these additional sources support the "negative" In vitro Bacterial Reverse Mutation Assay study results summarized above.

14.02.2006 (3) (5) (20) (21) (26) (31) (34) (35) (41) (58) (62) (73) (74) (75) (85) (86) (89) (108) (110) (115) (121) (132) (134) (137) (138) (143) (148) (155) (156) (163) (167) (187)

Remark : Data from these additional sources do not support the "negative" study results summarized above.

07.02.2006 (33) (72) (117) (182)

Remark : In vitro Unscheduled DNA Synthesis Test: No Data were available for MMF. See the following supporting data on DMF.

07.02.2006

Type : Unscheduled DNA synthesis
System of testing : Human embryonic intestinal cells
Test concentration : Highest concentration tested was 9.663 mg/L. Other concentrations were not reported.
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : other
Year : 1981
GLP : no data
Test substance : other TS

Method : No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

The test compound and positive controls were dissolved in dimethylsulfoxide (DMSO).

Positive control substances used in the test included 4 nitroquinoline-N-oxide and 2-aminoanthracene.

S9 mix contained NADP-di-Na-salt, glucose-6-phosphate-di-Na-salt, MgCl2, and KCl.

A preliminary toxicity test was conducted to establish the range of

concentrations to be used in the DNA repair assay. No toxicity was observed even at the highest concentration of 9.663 mg/mL which was selected as the highest in a series of concentrations of DMF.

Initial Assay: The cells were harvested, sedimented, and suspended in fresh culture medium at a density of 5x10E4 cells/mL, and samples of this suspension were pipetted into tissue culture Petri dishes containing 3 sterile coverslips. These were incubated at 37°C in a humid atmosphere of 5% CO2 in air for 72 hours. The medium was then replaced with arginine-deficient DMEM supplemented with 5% heat inactivated fetal bovine serum and the plates incubated for 24 hours. The medium was then replaced with an arginine-deficient DMEM and the incubation continued for 48 hours more. At the end of this time, the cultures were divided into 2 groups and S9 mix was added to one of the groups. Solutions of hydroxyurea in sterile distilled water and 6-[3H]-thymidine were added to each culture. DMF was dissolved in DMSO and dilutions were made from this stock solution to give the required concentrations. Triplicate wells, with and without S9, received samples of test compound solution. DMSO was added to the negative control cultures.

After incubation for 3 hours at 37°C in an atmosphere of 5% CO2 in air, the cultures were repeatedly rinsed in phosphate buffered saline (PBS), which removed loose cells and soluble [3H]-thymidine. They were then incubated for 10 minutes in sodium citrate and finally fixed in methanol:acetic acid for 18 hours. Coverslips were air dried and attached, cells uppermost, to clean microscope slides. Cells were then processed for autoradiography and stained. The stained autoradiographs were examined via microscope. Fifty nuclei were examined for each culture. The data were recorded as the average net grain counts for 3 coverslips ± the standard deviation.

Method 2: Flow 11,000 cells were harvested, sedimented, and suspended in fresh culture medium at a density of 5x10E4 cells/mL. Samples were dispensed into tissue culture Petri dishes which were incubated in a humid atmosphere of 5% CO2 in air at 37°C for 72 hours. The medium was then replaced with arginine-deficient medium supplemented with 5% heat-inactivated FBS and the dishes incubated for 24 hours. The medium was then replaced with an arginine-deficient DMEM and the incubation was continued for another 48 hours. The dishes were then randomly divided into 2 groups and S9 mix was added to one of the groups. Solutions of hydroxyurea and [3H]-deoxyguanosine were added to each dish. DMF was added to give a final concentration of 9.663 mg/mL.

After a 4-hour incubation at 37°C in an atmosphere of 5% CO2 in air, the cultures were washed 3 times with PBS, harvested using a trypsin/EDTA/solution and suspended in saline-EDTA. Cells were disrupted by 30 strokes of a glass pestle in a glass uniform homogeniser, NaCL and sodium lauryl sulfate added, and the mixture incubated for 10 minutes at room temperature. The lysate was then vigorously shaken with phenol-hydroxyquinoline and centrifuged for 15 minutes. The upper, aqueous phase was carefully removed and a sample was mixed with cesium chloride. The solution was poured into centrifuge tubes and overlaid with liquid paraffin. Tubes were centrifuged for 72 hours. Gradients were fractioned by upward displacement with saturated cesium chloride using an ISCO density fractionator, 8 drop fractions were collected on filter discs. The filter discs were immersed for 10 minutes in 2 changes of ice-cold trichloroacetic acid containing sodium pyrophosphate, washed twice in ice-cold hydrochloric acid, and finally once in ethanol. After air drying, the discs were placed in scintillation fluid and analyzed for radioactivity in a liquid scintillation counter. Gradient cells of the DNA from the cells treated with test compound were compared with the profiles of the DNA from cells incubated with 4-nitroquinoline-N-oxide, 2-aminoanthracene, and DMSO.

Several experiments were carried out in the presence of S9 mix in which it was found that no [3H]-deoxyguanosine was incorporated by any of the cultures treated. In the belief that some component of S9 was perhaps metabolizing the [3H]-deoxyguanosine to a derivative which was not incorporated into nucleic acids, the experimental protocol was altered. Cells which had been growing for 72 hours in arginine-deficient medium were treated with hyroxyurea, S9 mix, and test compound, 2-aminoanthracene, or DMSO. After a 3-hour exposure at 37°C, the incubation medium was removed and the cells were washed twice with PBS. The cells were then covered with new arginine-deficient medium. [3H]-deoxyguanosine was then added and the monolayers were incubated for a further 2.5 hours in the presence of the labeled precursor. The extraction procedure was the same as for those cells without S9.

Remark : Reliability: High because a scientifically defensible or guideline method was used.

Result : In the initial assay involving tritiated thymidine incorporation into non-S phase cells, there was no indication of any increase in the number of silver grains per nucleus at any concentration of DMF. Significant responses were observed in the cells treated with the positive controls.

The tritiated deoxyguanosine incorporation assay was used to confirm the results of the first assay. During the course of these experiments, the permeability of both cell lines to deoxyguanosine decreased dramatically. This reduction was aggravated by the addition of S9 mix to the incubation medium. Consequently, the measured incorporation of radioactivity was insufficient to provide a reasonable analysis of the data.

Test substance : DMF, purity 99%

07.02.2006 (141)

Remark : Data from these additional sources support the "negative" In vitro Unscheduled DNA Synthesis Test study results summarized above.

14.02.2006 (1) (31) (82) (95) (114) (118) (119) (142) (161) (173) (192) (193) (194)

Remark : In vitro Clastogenicity: No Data were available for MMF. See the following supporting data on DMF.

07.02.2006

Type : Cytogenetic assay
System of testing : Chinese Hamster Ovary (CHO) cells
Test concentration :
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : other
Year : 1992
GLP : yes
Test substance : other TS

Method : No specific test guideline was reported; however, the procedures used in the test followed those reported by Galloway, S. et al. (1985). Environ. Mutagen., 7:1-52 and Galloway, S. et al. (1987). Environ. Mol. Mutagen., 10(Suppl. 10):1-176.

CHO cells were incubated with DMF or solvent (DMSO) for induction of sister chromatid exchanges and chromosomal aberrations, both in the presence or absence of S9.

Statistical analyses were conducted on both the slopes of the dose-response curves and the individual dose points. An SCE frequency that

was 20% above the concurrent solvent control value was chosen as a statistically positive result. A single increased dose was considered weak evidence for a positive response. A positive response occurred if 2 or more doses were significantly increased.

Chromosomal aberration data are presented as the percentage of cells with aberrations. The dose-response curve and individual dose points were statistically analyzed. For a single trial, a statistically significant difference for 1 dose point and a significant trend were considered weak evidence for a positive response. Significant differences for 2 or more doses indicated the trial was positive.

For the SCE study, 2 trials were conducted with and without S9 activation with a total of 50 cells analyzed per concentration in each of the trials. In the chromosome aberration study, 1 trial was conducted with and without activation with a total of 100 cells analyzed per concentration.

Mitomycin C and cyclophosphamide were tested as positive control substances.

Remark : Reliability: High because a scientifically defensible or guideline method was used.

Result : The expected positive results were observed in the cells treated with the positive controls.

Test condition : SCE Trial 1: 0, 50, 160, 500, 1600, 5000 µg/mL (-S9)
SCE Trial 2: 0, 1600, 3000, 4000, 5000 µg/mL (-S9)
SCE Trial 1: 0, 160, 500, 1600, 5000 µg/mL (+S9)
SCE Trial 2: 0, 100, 1600, 3000, 4000, 5000 µg/mL (+S9)
Chromosome aberration: 0, 1600, 3000, 4000, 5000 µg/mL (-S9)
Chromosome aberration: 0, 1000, 1600, 3000, 5000 µg/mL (+S9)

Test substance : DMF, purity > 99%

14.02.2006 (143)

Remark : Data from these additional sources support the "negative" In vitro Clastogenicity study results summarized above.

14.02.2006 (3) (20) (23) (32) (52) (55) (56) (84) (129) (130) (136) (139) (140) (143) (150) (153) (157) (169) (170)

Remark : Data from these additional sources do not support the "negative" study results summarized above.

07.02.2006 (33) (97) (117)

Remark : Data from these additional sources support the "negative" study results summarized above. References include cell transforming studies, DNA repair, and aneuploidy studies.

07.02.2006 (80) (119) (120) (151) (152) (153) (156) (157) (175) (177) (189)

Remark : Data from these additional sources do not support the "negative" study results summarized above. References include cell transforming studies, DNA repair, and aneuploidy studies.

07.02.2006 (29) (80) (147) (149) (155) (168)

5.6 GENETIC TOXICITY 'IN VIVO'

Remark : In vivo Mouse Micronucleus Assay: No Data were available for MMF. See the following supporting data on DMF.

06.02.2006

Remark : Supporting Data for Dimethylformamide (DMF)
06.02.2006

Type : Micronucleus assay
Species : mouse
Sex : male
Strain : Balb/c
Route of admin. : i.p.
Exposure period :
Doses : 0, 0.2, 20, 2000 mg/kg
Result : negative
Method : other
Year : 1983
GLP : no data
Test substance : other TS

Method : No specific test guideline was reported; however, procedures generally followed those outlined in Schmid. W. (1976). "The micronucleus test for cytogenetic analysis" in Hollaender. A. (Ed). Chemical Mutagens, Vol. 4, pp. 31-53, Plenum, New York.

Mice were 12-13 weeks old at study start. Five animals were used in each treatment group. Positive controls received 100 mg/kg cyclophosphamide. Preparations were made 30 hours after treatment.

Remark : Reliability: Medium because a scientifically defensible or guideline method was used; however, limited study information was available.

Result : The number of cells with micronuclei in each test group are as follows [Cells with micronuclei (%±S.D.)]: Negative control, 1.6±0.24; Cyclophosphamide, 44.2±1.6; DMF 0.2 mg/kg, 1.8±0.37; DMF 20 mg/kg, 1.6±0.40; and DMF 2000 mg/kg, 1.8±0.37

Test substance : DMF, purity not reported
07.02.2006 (3)

Remark : Data from these additional sources support the "negative" Micronucleus assay study results summarized above.
14.02.2006 (93) (94) (165) (166) (183) (184)

Remark : Data from these additional sources do not support the "negative" study results summarized above.
06.02.2006 (196)

Remark : In vivo Dominant Lethal Assay: No Data were available for MMF. See the following supporting data on DMF.
06.02.2006

Remark : Supporting Data for dimethylformamide (DMF).
06.02.2006

Type : Dominant lethal assay
Species : rat
Sex : male
Strain : Sprague-Dawley
Route of admin. : inhalation
Exposure period : 6 hours a day for 5 days
Doses : 0, 30, and 300 ppm

Result : negative
Method : other
Year : 1978
GLP : no
Test substance : other TS

Method : No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

There were 10 male rats per group.

Each male was caged with 2 females per week for 2 consecutive weeks during the pre-treatment mating period. The females were sacrificed 18 days after the first day of caging with the males and implants observed to indicate pregnancy. Males were considered fertile if at least 1 female was pregnant.

For each DMF chamber, a stream of dry air at room temperature was passed through a bubbler containing DMF. The air flow rate was monitored with a rotameter and was varied to volatilize the appropriate amount of DMF. The vapor-air mixture was diluted to the appropriate concentration with room air prior to entering the exposure chamber. The chamber flow rates were also varied to maintain the proper concentration. Exposure chambers, operated dynamically, were stainless steel and glass with an effective volume of 760 L. Atmospheric sampling was conducted and analyzed via an infrared analyzer.

On the last day of the treatment period, at least 2 hours after the last exposure, 2 untreated virgin females were placed in to each male's cage. The male and females remained together for 7 days at which time the females were removed and replaced with 2 new, untreated virgin females. This mating procedure continued for 6 consecutive weeks (post-treatment period). Females removed from the male's cages were sacrificed 18 days after the first day of caging with the male, and implantation data were recorded. Females were considered pregnant when at least 1 uterine implantation site was observed during the gross examination. The number of uterine implantation sites was recorded as well as early resorption sites, late resorption sites, and viable fetal swellings.

Males were sacrificed at the completion of the study following sacrifice of the last females and a necropsy was performed. For 5 males/group, the seminal vesicles, epididymides, prostate, and testes were preserved and examined histopathologically. Any abnormal lesions or tissue masses observed on any male were also preserved and examined.

TEM (triethylenemelamine) was used as the positive control substance in the study. The males in the positive control group received a single i.p. dose of 0.3 mg/kg 2 hours prior to mating.

Comparisons were made for each post-treatment mating week between the negative control, positive control, and DMF-treated groups. Incidence data were evaluated by the Chi-square test. Absolute data were compared using the F-test and Student's t-test. When variances differed significantly, Student's t-test was appropriately modified using Cochran's approximation.

Remark : Reliability: High because a scientifically defensible or guideline method was used.

Result : There was no mortality observed during the study. Pharmacologic and toxicologic observations recorded during the study showed no test-substance related findings.

At the 300 ppm level, DMF-treated males had a slightly lower mean body weight gain during the post-treatment period.

Pregnancy rates and implantation efficiency values for females exposed to DMF-treated males were comparable to the negative control throughout the post-treatment period.

Fetal death data (mean per pregnant female and percentage of total implantation sites) for the DMF-treated groups were slightly higher than control at week 2 (both groups), week 5 (30 ppm group), and week 6 (300 ppm group). At each interval, the increase in fetal deaths in the DMF-treated groups was, in part, attributed to a single female that had uterine implants comprised entirely of early fetal deaths. These increases in fetal deaths were not considered indicative of a dominant lethal mutagenic response since the second female of the pair mated with the DMF-treated male had high numbers of uterine implants which in most cases were all viable fetal swellings.

There were no treatment-related histopatholgic alterations noted during the study.

Test substance : DMF, purity not reported
14.02.2006

(17) (28) (104)

Remark : Data from these additional sources support the "negative" in vivo Dominant lethal assay study results summarized above.
14.02.2006

(6) (7) (8) (77) (78) (79) (116) (133) (142) (172)

Remark : Data from these additional sources of other in vivo genetic toxicity studies support the "negative" study results summarized above.
14.02.2006

(2) (53) (54) (59) (79) (116) (142) (143) (145) (146) (154) (155) (156) (180) (181) (195) (197)

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	other: Crl:CD (SD)COBS rats and New Zealand White rabbits
Sex	:	female
Strain	:	
Route of admin.	:	gavage
Exposure period	:	Rats/Gestation days 6-15 and Rabbits/Gestation days 6-18
Frequency of treatm.	:	Daily
Duration of test	:	
Doses	:	Rats/0, 1, 5, 10, 75 mg/kg and Rabbits/0, 5, 10, 50 mg/kg
Control group	:	yes
NOAEL maternal tox.	:	10 mg/kg bw
NOAEL teratogen.	:	10 mg/kg bw
Method	:	other
Year	:	1989
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4

Method : The procedures used in the test were based on the recommendations of the following guideline: EPA (1986). Developmental toxicity risk assessment guidelines, Fed. Regist., 51:34028-34040.

All animals were housed individually in stainless steel cages suspended over cage board in environmentally controlled rooms. Food and tap water were available ad libitum. A-12 hour light/12-hour dark photoperiod was provided. Room temperature was maintained at 72±4°F for rats and 68±5°F for rabbits. Relative humidity was maintained at 55±15%.

Female rats (226-289 g) were naturally mated with adult males from the same strain. The presence of a copulatory plug or a positive vaginal smear was designated as gestation day 0.

Female rabbits (ca. 3-4.1 kg) were artificially inseminated. Human chorionic gonadotropin was administered to the rabbits in the marginal ear vein immediately following the insemination. The day of insemination was designated as gestation day 0.

There were 25 pregnant female rats and 20 pregnant female rabbits per test group.

Dose suspensions were prepared in deionized water every 3 days for rats and every 7 days for rabbits. Animals in the control group received deionized water.

Maternal body weights and physical signs were monitored throughout the study. Food consumption was measured on designated weigh days (rats) or daily (rabbits).

Cesarean sections were performed on rats and rabbits on gestation days 20 and 29, respectively. Uterine and fetal examinations were conducted.

Individual rat fetuses were weighed and examined externally for anatomical anomalies and sex determination. Approximately half of the fetuses from each litter underwent a visceral examination by the method of Wilson, J. G. (1965). In Teratology Principles and Techniques, University of Chicago Press, Chicago. The remaining fetuses were eviscerated, fixed in ethanol, and stained with alizarin red S for skeletal evaluation.

All rabbit fetuses were individually weighed and examined for external and visceral anomalies by the method of Staples, R. E. (1974). Teratology, 9:A37-A38. Sex was determined during the internal examination. Rabbit fetuses were examined for cephalic soft tissue anomalies by a mid-cranial section. Rabbit fetuses were fixed in ethanol and stained with alizarin red S for skeletal examination.

Continuous data including fetal and maternal body weights, maternal body weight gain, maternal food consumption, number of fetuses, implantations, and corpora lutea were analyzed via one-way analysis of variance. When appropriate, group comparisons were conducted using Dunnett's analysis. Discontinuous data were analyzed using the chi-square test for fetal sex ratios, Mann-Whitney U test for resorptions, and a one-tailed Fisher exact test for the number of fetal variations and malformations.

Remark : Reliability: High because a scientifically defensible or guideline method was used.

Result : Dose suspensions were determined analytically by gas chromatography to be accurately prepared, homogeneous, and stable for 7 days at room temperature.

Rats

There were no treatment-related maternal deaths, clinical signs, or gross pathologic changes in treated dams. Body weight gain and food consumption were depressed in rats given 75 mg/kg.

GDay 0-20 data:

Dose (mg/kg)	Weight gain	Food consumption
0	151	24
1	147	25
5	143	24
10	145	24
75	113*	22*

* = significantly different from the control group by one-way analysis of variance, p<0.01.

The number of corpora lutea, implantations, and fetal sex ratio were similar in all experimental groups. Fetal viability was reduced at 75 mg/kg in rats due to a significant increase in the number of early resorptions. Fetal weights were depressed in the 75 mg/kg group. A summary of reproductive outcomes (means/litter unless otherwise noted) is provided in the table below:

Observation [0, 1, 5, 10, and 75 mg/kg, respectively]:

No. gravid females with live litter: 25, 25, 25, 25, and 22

Corpora lutea/dam: 17.7, 17.3, 16.5, 16.6, and 16.5

Pre-Implantation loss/litter: 2.0, 1.2, 1.6, 0.6, and 1.1

Post-Implantation loss/litter: 0.9, 1.0, 0.8, 1.0, and 3.1*

Total number of live fetuses: 370, 375, 351, 373, and 269

Live Fetuses/litter: 14.8, 15.0, 14.0, 14.9, and 12.2**

Mean Fetal Weight (g): 3.5, 3.5, 3.5, 3.4, and 2.9**

Sex Ratio (M/F): 204/166, 196/179, 178/173, 185/188, and 127/142

Number of malformed fetuses/number of litters affected: 0/0, 0/0, 1/1, 3/3, 150/21***

* = significantly different from the control group by Mann-Whitney U test, P<0.01
** = significantly different from the control group by one-way analysis of variance, P<0.01
*** = significantly different from the control group by Fisher's exact test, P<0.05

At 75 mg/kg, over 50% of the fetuses were malformed. A significant increase in the incidence of malformations including cephalocele and sternoschisis was observed in fetuses from the 75 mg/kg group. The isolated incidences of the malformations of gastroschisis, right-sided aortic arch, and sternebrae fused at 75 mg/kg were considered to be spontaneous in nature.

In addition, a developmental delay was indicated by reduction of fetal weight and by a significant increase in the occurrence of incomplete ossification of various skeletal structures, including reduced ossification of general non-ossification of the skull, 13th rib(s), and sternebrae.

Fetal data from the 1, 5, and 10 mg/kg groups were comparable to the

control group. There were 3 fetuses malformed in the 10 mg/kg group; however, these malformations were considered to be spontaneous in nature because of the single incidence and lack of a dose-response relationship. The number of fetuses/litter with reduced ossification of the 13th rib was slightly increased at 10 mg/kg compared to control values. However, the variation occurred at an incidence comparable to the historical control population of the testing facility. For the historical control population, 0.0 to 3.6% of the fetuses were affected.

A summary of fetal malformations and variations in rats is provided in the table below. Incidences of findings are presented as number of fetuses affected/number of litters affected unless otherwise indicated [0, 1, 5, 10, and 75 mg/kg, respectively].

External Exam*: 370/25, 375/25, 351/25, 373/25, and 269/22
Visceral Exam*: 188/25, 189/25, 179/25, 185/25, and 134/22
Skeletal Exam*: 182/25, 187/25, 172/25, 189/25, and 135/22

* = Number of fetuses examined/number of litters

Malformations

Cephalocele: 0/0, 0/0, 0/0, 0/0, and 144/20**

Sternoschisis: 0/0, 0/0, 0/0, 0/0, and 9/5**

Anal atresia and filamentous tail: 0/0, 0/0, 0/0, 1/1, and 0/0

Gastroschisis: 0/0, 0/0, 0/0, 0/0, and 1/1

Micrognathia: 0/0, 0/0, 0/0, 1/1, and 0/0

Open eyelids: 0/0, 0/0, 0/0, 1/1, and 0/0

Multiple anomalies: 0/0, 0/0, 0/0, 1/1, and 0/0

Right-sided aortic arch: 0/0, 0/0, 0/0, 0/0, and 1/1

Fused sternebrae: 0/0, 0/0, 0/0, 0/0, and 1/1

Vertebral anomalie: 0/0, 0/0, 1/1, 0/0, and 0/0

Atlas-occipital defect: 0/0, 0/0, 0/0, 1/1, and 0/0

Variations

Reduced ossification of the skull: 8/5, 7/4, 5/3, 5/3, and 66/15**

Unossified sternebrae no. 5 and/or no. 6: 6/3, 6/5, 6/5, 13/5, and 36/13**

Reduced ossification of 13th rib: 2/2, 3/2, 0/0, 8/6, and 12/8**

Unossified sternebrae nos. 1, 2, 3, and/or 4: 0/0, 1/1, 0/0, 1/1, and 5/5**

Distended ureter: 7/4, 15/11, 13/8, 12/6, and 19/10

Malaligned sternebrae: 38/21, 48/21, 38/20, 55/24, and 58/19

14th rudimentary rib: 6/4, 2/2, 3/3, 2/1, and 0/0

Bent ribs: 4/4, 2/2, 4/3, 0/0, and 1/1

7th cervical rib: 4/3, 0/0, 0/0, 3/3, and 0/0

Sternebrae with thread-like attachment: 0/0, 0/0, 0/0, 0/0, and 1/1

27 presacral vertebrae: 0/0, 0/0, 1/1, 0/0, and 0/0

Unossified pubis: 0/0, 0/0, 0/0, 0/0, and 1/1

Unossified hyoid: 1/1, 1/1, 0/0, 0/0, 1/1

** = Significantly different from the control group by Fisher's exact test, P<0.05.

Rabbits

There were no treatment-related maternal deaths, clinical signs, or gross pathologic changes in treated dams. Maternal deaths of 1 female in the 5 and 10 mg/kg groups, and 2 females in the 50 mg/kg group were the result of dosing trauma. No other deaths occurred. Body weight gain and food consumption were depressed in rabbits given 50 mg/kg.

GDay 0-29 data:

Dose (mg/kg)	Weight gain	Food consumption
0	573	164
5	616	172
10	489	165
50	448	145

The mean number of corpora lutea was reduced at 50 mg/kg; however, the authors considered this reduction to be of no toxicological significance because the mean number of corpora lutea in the control group exceeded the historical control range for the testing facility (mean 12.0, range, 10.9-12.3). In addition, historical control data from MARTA for this parameter has been determined to be 10.5 for New Zealand white rabbits (Lang, P. L. (ed). (1993). Historical Control Data for Developmental and Reproductive Toxicity Studies Using the New Zealand White Rabbit, Hazleton Research Products, Inc., Denver, Pa.).

The number of implantations and sex ratio were unaffected by treatment. Fetal viability was reduced at 50 mg/kg in rabbits as evidenced by post-implantation loss. Fetal body weight was also reduced at 50 mg/kg. A summary of reproductive outcomes (means/litter unless otherwise noted) is provided in the table below:

Observation [0, 5, 10, and 50 mg/kg, respectively]:

No. gravid females with live litter: 19, 17, 16, and 15

Corpora lutea/doe: 13.4, 11.9, 10.9, and 9.6*

Pre-Implantation loss/litter: 6.8, 4.9, 4.2, and 3.2

Post-Implantation loss/litter: 0.4, 0.5, 0.5, and 1.5**

Total number of live fetuses: NR, NR, NR, and NR

Live Fetuses/litter: 6.2, 6.5, 6.2, and 5.0

Mean Fetal Weight (g): 46.1, 44.2, 46.1, and 37.0

Sex Ratio (% M/F): 53/64, 50/60, 50/49, and 38/47

No. of malformed fetuses/number of litters affected: 5/5, 4/3, 3/3, 85/15***

NR = Not Reported
* = significantly different from the control group by one-way analysis of variance
**= significantly different from the control group by Mann-Whitney U test, P<0.05
*** = significantly different from the control group by Fisher's exact test, P<0.05

Treatment-related malformations observed at 50 mg/kg included gastroschisis, cephalocele, domed head, flexed paw, and skull and sternum anomalies. Although there was an increased incidence of the developmental variation of bent hyoid at 50 mg/kg, this finding was not statistically significant. Fetal data for the 5 and 10 mg/kg were comparable to control data. A summary of fetal malformations and variations in rabbits is provided in the table below. Incidences of findings are presented as number of fetuses affected/number of litters affected unless otherwise indicated [0, 5, 10, and 50 mg/kg, respectively].

External Exam*: 117/19, 110/17, 99/16, and 85/15
Visceral Exam*: 117/19, 110/17, 99/16, and 85/15
Skeletal Exam*: 117/19, 110/17, 99/16, and 85/15

* = Number of fetuses examined/number of litters

Malformations

Gastroschisis: 0/0, 0/0, 0/0, and 84/15**

Cephalocele: 0/0, 0/0, 0/0, and 14/5**

Domed head: 0/0, 0/0, 0/0, and 11/4**

Skull anomaly: 0/0, 0/0, 0/0, and 46/12**

Sternum anomaly: 0/0, 0/0, 0/0, and 75/14**

Micrognathia: 1/1, 0/0, 0/0, and 0/0

Flexed paw: 0/0, 0/0, 0/0, and 10/3

Hydrocephaly: 1/1, 0/0, 0/0, and 0/0

Kidney and/or ureter agenesis: 0/0, 0/0, 0/0, and 1/1

Rib anomaly: 1/1, 0/0, 0/0, and 0/0

Sternebrae fused: 1/1, 2/2, 1/1, and 3/1

Sternebrae misaligned (severe): 0/0, 0/0, 1/1, and 0/0

Vertebral anomaly: 2/2, 2/1, 0/0, and 1/1

Extra site of ossification anterior to sternebra no. 1: 0/0, 0/0, 1/1, and 0/0

Variations

Major blood vessel variation: 3/3, 0/0, 1/1, and 1/1

Hemorrhagic ring around the iris: 3/3, 3/3, 1/1, 0/0

Retrocaval ureter: 5/4, 2/2, 1/1, 3/3

Gall bladder absent or small: 0/0, 1/1, 0/0, and 2/2

13th full rib: 55/16, 43/15, 54/15, and 51/13

Sternebrae no.5 and/or no. 6 unossified: 13/4, 3/3, 3/1, and 1/1

13th rudimentary rib: 14/9, 17/12, 10/6, and 17/10

27 presacral vertebrae: 22/11, 10/8, 21/10, and 23/6

Sternebrae with threadlike attachment: 1/1, 2/2, 4/3, and 1/1

Hyoid arches bent: 6/6, 8/5, 3/3, and 22/9

Sternebrae malaligned (slight or moderate): 19/11, 16/8, 11/8, and 3/3**

Accessory skull bone: 0/0, 2/2, 0/0, and 1/1

Hyoid body and/or arches unossified: 0/0, 1/1, 0/0, and 0/0

7th sternebrae: 0/0, 0/0, 2/2, and 0/0

* = Number of fetuses examined/number of litters
** = significantly different from the control group by Fisher's exact test, P<0.05

The lowest-observed-adverse-effect levels for maternal and developmental toxicity in the rat and rabbit were 75 and 50 mg/kg, respectively. The no-observed-adverse-effect level for maternal and developmental toxicity in the rat and rabbit was 10 mg/kg.

Test substance : N-methylformamide, purity greater than 99.9%
15.02.2006 (90) (107)

Species	:	rat
Sex	:	female
Strain	:	other: Crl:CD®BR
Route of admin.	:	inhalation
Exposure period	:	Gestation Days 7-16
Frequency of treatm.	:	6 hours/day
Duration of test	:	
Doses	:	0, 15, 50, 150 ppm
Control group	:	yes
NOAEL maternal tox.	:	15 ppm
NOAEL teratogen.	:	15 ppm
Method	:	other
Year	:	1988
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method : The procedure used in the test were based on the recommendations of the following guidelines:

US EPA (1985). TSCA Test Guidelines, Federal Register, 50, No. 188, September 27; US EPA (1987). Revised 52, No. 97, May 20 and

US EPA (1984). New and Revised Health Effects Test Guidelines, October, US EPA, Washington, DC.

Female rats were 63 days old upon arrival and weighed between 170.1 to 218.3 grams on the day after arrival. Mature male rats of the same strain and from the same supplier were 84 days old upon arrival and weighed between 308.9 to 376.4 grams prior to mating. Animals were individual housed, except during mating, in stainless steel wire-mesh cages. Food and water were available ad libitum. Animal rooms were on a 12-hour light/12-hour dark cycle. Room temperature and relative humidity were targeted at 21-25°C and 40-60%, respectively.

Females were cohabited with males (1:1) until copulation was confirmed by the presence of a copulatory plug in the vagina or on the cageboard. The day that copulation was confirmed was designated as day 1 of gestation.

There were 25 pregnant female rats per test group.

Test atmospheres of MMF were generated by vaporization. Conditioned, filtered houseline air was metered through glass reservoirs containing the liquid test material to generate MMF vapor. The reservoirs for the intermediate and high-level chambers were immersed in water baths and heated to approximately 21-29°C to increase the evaporation rate of the test material. The reservoir for the low level chamber was maintained at ambient temperature. Conditioned, filtered houseline air was added to dilute the vapor and to sweep the resulting vapor/air mixtures into the inlets of 150-L stainless steel and glass exposure chambers. Exposure chamber concentrations of MMF were controlled by varying the flow rates of generation or dilution air. To promote uniform distribution, the test mixtures were dispersed with baffles upon entering the exposure chambers. The control rats were exposed to conditioned, filtered houseline air only, using the same type of exposure chamber.

The atmospheric concentration of MMF in each test chamber was measured periodically by gas chromatographic analysis. Chamber temperatures, relative humidity, and chamber oxygen concentrations were also measured.

Rats were individually restrained in perforated, stainless steel cylinders fitted with conical nose pieces. The nose pieces were coated with parafilm to minimize droplet deposition on the rats' face or head. Each restrainer was inserted into a Plexiglas face plate on the exposure chamber such that only the rat's nose and potentially part of the head could protrude into the chamber.

Dams were regularly monitored throughout gestation for body weight gain, feed consumption, and clinical signs. Cesarean sections were performed on Day 22G and the adult females were examined for gross anatomical abnormalities. Liver, thymus, and gravid uterus were removed and weighed. Additional parameters assessed included the number and relative position of nidations (live and dead fetuses, and early and late resorptions), empty uterine weight, and the number of corpora lutea.

Fetal weights and fetal sex were recorded. An external examination was conducted on all fetuses. Approximately half of the fetuses/litter were decapitated and examined for visceral examinations using the Staples technique (Staples, R. E. (1974). Teratology, 9:A37). The heads were fixed in Bouin's solution and examined. All fetuses underwent a skeletal examination.

Incidence of pregnancy, clinical observations, maternal mortality, and litters with total resorptions were analyzed via the Cochram-Armitage test for linear trend and the Fisher's exact test. Maternal weight, weight change, feed consumption, and maternal liver and thymus weights were analyzed with the linear combination of dose ranks from ANOVA and the Dunnett's

test if the ANOVA was significant. Live fetuses, dead fetuses, resorptions, nidations, corpora lutea, fetal weight, and incidence of fetal alterations were analyzed with Jonkheere's test and the Mann-Whitney U test.

Remark : Reliability: High because a scientifically defensible or guideline method was used.

Result : The mean analytical concentrations for the 0, 15, 50, and 150 ppm groups were 0, 14.8, 51.5, and 150 ppm, respectively. The chamber temperatures ranged from 21-28, 23-29, 24-30, and 24-29°C for the 0, 15, 50 and 150 ppm concentration levels, respectively. The mean percent relative humidity was 38-58, 44-68, 45-60, and 44-61% for the 0, 15, 50, and 150 ppm concentration groups, respectively. The chamber oxygen concentrations were approximately 21% for both test and control chambers throughout the study.

One compound-related death occurred on day 14 of gestation in the 150 ppm group. Exposure to 50 or 150 ppm produced significant increases in the number of dams exhibiting mild respiratory distress, characterized as wheezing and rattling, during and after the exposure period. Other clinical signs observed were primarily attributable to the restraint conditions of the study, however, a possible decrease in grooming at 150 ppm may explain the persistence of some of these signs post-exposure. Maternal toxicity was also evident at the 150 ppm level as evidenced by significant adverse effects on body weight, food consumption, and absolute and relative thymus weights.

Embryolethality was evident by the significantly increased mean number of resorptions per litter seen in females exposed to 150 ppm of MMF. Correspondingly, the mean number of live fetuses per litter was significantly decreased at 150 ppm. Developmental toxicity was also evident as a significant reduction in mean fetal body weight at exposure levels of 50 and 150 ppm; a significant trend accompanied this finding. Relative to controls, the weight reduction at 50 ppm was regarded as slight. A summary of reproductive outcomes (means/litter unless otherwise noted) is provided in the table below [0, 15, 50, and 150 ppm groups, respectively]:

Observation

Number pregnant: 23/25, 25/25, 24/25, and 22/25

Corpora lutea: 15.3, 15.6, 15.1, and 15.4

Nidations: 14.5, 14.2, 13.6, and 14.2

Total No. of Fetuses: NR, NR, NR, and NR

Mean Resorptions: 0.9 T/0.9 E/0.0 L, 0.6 T/0.6 E/0.0 L, 0.8 T/0.8 E/0.0 L, and 2.0 T*/2.0 E*/0.0 L

Live Fetuses: 13.7 T/6.9 M/6.7 F, 13.6 T/6.9 M/6.7 F, 12.8 T/6.5 M/6.3 F, and 12.3 T*/6.6 M/5.7 F*

Mean Fetal Weight (g): 5.17 T/5.30 M/5.00 F, 5.12 T/5.23 M/5.02 F, 5.06 T/5.20 M/4.86 F, and 4.54 T*/4.65 M*/4.40 F*

Sex Ratio (No. of Males/Total No.): NR, NR, NR, and NR

T = total, E = early, L = late
M = male, F = female
NR = Not Reported
* = significantly different from controls (Mann-Whitney U test), p equal to or less than 0.05.

The incidence of structural malformations was significantly increased in fetuses from the 150 ppm exposure group. The malformations that primarily contributed to this increase were subcutaneous head cysts, microphthalmia, hydrocephaly, distended ventricles of the brain, fused ribs and vertebrae, and hemivertebrae. Additionally, developmental toxicity was evident by an exposure-related response in the incidence of fetuses with variations, specifically those due to growth retardation where a significant delay in skeletal ossification was seen in fetuses from the 150 ppm group. Although the overall incidence of developmental variations was not significantly different across groups, significance was noted for total skeletal variations consisting primarily of misaligned and fused sternebrae. A significant trend and increase relative to control values was also seen in the 150 ppm group for the mean percentage of fetuses per litter with variations due specifically to retarded development. This effect was attributable to delayed skeletal ossification. A summary of fetal malformations and variations in rats is provided in the table below. Incidences of findings are presented as number of fetuses affected/number of litters affected unless otherwise indicated [0, 15, 50, and 150 ppm groups, respectively].

External Exam*: 314/23, 341/25, 308/24, and 258/21
Visceral Exam*: 164/23, 177/25, 160/24, and 148/21
Skeletal Exam*: 314/23, 341/25, 307/24, and 258/21

* = Number of fetuses examined/number of litters

Malformations

Abdomen - Gastroschisis: 0/0, 0/0, 0/0, and 1/1

Head - Subcutaneous cyst: 0/0, 0/0, 0/0, and 21/2

Tail - Vestigial: 0/0, 0/0, 1/1, and 0/0

Kidney - No papilla: 0/0, 1/1, 0/0, and 0/0

Brain - 3rd ventricle distended: 0/0, 0/0, 0/0, and 1/1

Brain - Hydrocephaly: 1/1, 0/0, 0/0, and 2/2

Brain - Lateral ventricle distended: 0/0, 2/2, 1/1, and 4/2

Eye - Anophthalmia: 0/0, 0/0, 0/0, and 1/1

Eye - Microphthalmia: 0/0, 0/0, 0/0, and 3/1

Rib - Fused: 0/0, 0/0, 0/0, and 9/2

Vertebrae - Fused: 0/0, 0/0, 0/0, and 7/1

Vertebrae - Hemi: 0/0, 0/0, 0/0, and 8/1

Total No. Affected with Malformations: 1/1, 3/3, 2/2, and 30/6**

Variations

Sternebra - Misaligned (1): 1/1, 3/2, 2/2, and 2/2

Sternebrae - Misaligned (2+): 1/1, 2/2, 2/2, and 11/6

Sternebra - Fused: 0/0, 0/0, 0/0, and 13/4

Total No. Affected with Skeletal Developmental Variations: 27/12, 15/10, 11/6, and 32/15

Mean % Affected: 7.8, 4.3, 4.6, and 18.7**

Rib - Rudimentary Th13: 6/5, 4/4, 8/5, and 28/10

Skull - Frontal partially ossified: 0/0, 0/0, 0/0, and 7/6

Skull - Interparietal partially ossified: 9/6, 13/7, 12/11, and 32/12

Skull - Parietal partially ossified: 0/0, 7/4, 3/3, and 12/5

Skull - Squamosal partially ossified: 2/2, 4/3, 2/2, and 8/4

Skull - Supraoccipital partially ossified: 2/2, 10/6, 8/8, and 87/19

Skull - Supraoccipital unossified: 0/0, 0/0, 0/0, and 9/4

Skull - Zygoma partially ossified: 3/3, 6/5, 3/3, and 9/4

Vertebra - Dumbbelled centrum: 8/8, 8/5, 16/8, and 26/12

Vertebra - Partially ossified: 2/2, 2/2, 8/6, and 20/11

Total No. with Variations due to Retarded Development: 44/20, 49/17, 59/22, and 137/20

Mean % Affected: 13.3, 13.8, 19.4, and 62.0**

** = significantly different from the controls (Mann-Whitney U test), P equal to or less than 0.05

Under the conditions of this study, maternal lethality and toxicity was demonstrated at 150 ppm of MMF and maternal toxicity remained evident as mild respiratory distress in the 50 ppm treated dams. Although to a lesser degree than at 150 ppm, developmental toxicity, expressed as a slight depression in mean fetal body weight was evident at 50 ppm. The NOEL for both the dam and the fetus was 15 ppm. Thus the conceptus was found to be sensitive only at exposure levels that were also toxic to the dams.

Test substance : N-methylformamide, purity 99.9%

14.02.2006 (47) (158) (159)

Remark : MMF has been studied for its developmental toxicity by the oral, inhalation, dermal, intraperitoneal, subcutaneous, and in vitro routes of exposure. The 2 studies which represented the most standard study designs for developmental toxicity and which followed recommended EPA test guidelines were the studies chosen for detailed summarization. Of these studies, one was via the oral route of exposure and one was via the inhalation route of exposure. Both studies determined that the conceptus was sensitive to MMF only at exposure levels that were also toxic to the dams.

03.02.2006

Remark : Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Oral administration
03.02.2006 (46) (122)

Remark : Dermal administration
07.02.2006 (51) (176)

Remark : Data from these additional sources were not summarized because insufficient information was available. These studies reported fetal effects; however, no information on maternal toxicity was reported.

Oral administration
03.02.2006 (36)

Remark : Oral, intraperitoneal, and dermal administration
07.02.2006 (179)

Remark : Dermal administration
07.02.2006 (51) (60) (171) (176)

Remark : Subcutaneous administration
07.02.2006 (191)

Remark : Intraperitoneal administration
07.02.2006 (60)

Remark : Data from these additional sources were not summarized because insufficient information was available. These studies reported differential toxicity between the fetus and the mother; however, limited data were reported.

Oral administration
03.02.2006 (178)

Remark : Oral and dermal administration
07.02.2006 (162)

Remark : Intraperitoneal administration
07.02.2006 (98) (99) (191)

Remark : Oral, intraperitoneal, and dermal administration
07.02.2006 (144)

Remark : Dermal administration
07.02.2006 (38) (39) (185)

Remark : Data from this additional source were not summarized because the result was inconsistent with the majority of the other findings.
03.02.2006 (100)

Remark : Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the study design was in vitro rather than in vivo and the data were not substantially additive to the database.

03.02.2006 (63)

Remark : Data from these sources were not presented because the study design was in vitro and did not support existing in vivo data.

03.02.2006 (87) (88) (96)

Remark : Supporting Data for Dimethylformamide (DMF)
03.02.2006

Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	Gestation days 6-20
Frequency of treatm.	:	Daily
Duration of test	:	
Doses	:	0, 50, 100, 200, 300 mg/kg
Control group	:	yes
NOAEL maternal tox.	:	50 mg/kg bw
NOAEL teratogen.	:	50 mg/kg bw
Method	:	other
Year	:	1997
GLP	:	no data
Test substance	:	other TS

Method : No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

Time-mated rats were used on the study. Females had been housed overnight with adult males (1 male: 2-3 females) from the same strain). The day sperm was detected in the vaginal smear was considered to be gestation day 0. Mated females were housed singly.

There were 22-24 pregnant females per test group.

The control group received the distilled water vehicle.

Females were observed daily for clinical signs of toxicity. Food consumption and body weights were periodically measured. Rats were killed on gestation day 21 and the uterus was removed and weighed. Uterine contents were examined to determine the number of implantation sites, resorptions, and dead and live fetuses. Live fetuses were weighed, sexed, and examined for external anomalies including those of the oral cavity. Half of the live fetuses from each litter were preserved in Bouin's solution and examined for internal soft tissue changes via the techniques of Wilson and Barrow and Taylor. The other half of the fetuses in each litter were eviscerated and processed for skeletal examination.

The number of implantation sites and live fetuses and various body weights were analyzed by one-way analysis of variance, followed by Dunnett's test if differences were found. The frequency of resorptions and anomalies among litters was evaluated by using the Kruskal-Wallis test followed by the Dixon-Massey test where appropriate. Rates of pregnancy and fetal sex ratio were analyzed by using Fisher's test.

Remark : Reliability: High because a scientifically defensible or guideline method was

used.

Result : All females survived to scheduled termination. Maternal weight gain, maternal body weights, and food consumption were significantly reduced in the 100, 200, and 300 mg/kg groups.

Fetal body weight per litter was significantly reduced at 100 mg/kg and higher. A summary of reproductive outcomes (means/litter unless otherwise noted) is provided in the table below [0, 50, 100, 200, and 300 mg/kg/day dose groups, respectively].

% Pregnant: 66.7, 95.5, 86.4, 86.4, and 90.9

No. of litters examined: 16, 21, 19, 19, and 20

Mean implantation sites: 15.81, 14.48, 15.47, 15.53, and 15.25

Mean live fetuses: 15.25, 13.81, 14.79, 14.58, and 14.05

Mean % resorption sites: 3.71, 8.62, 4.45, 6.15, and 7.55

Fetal sex ratio (M/F): 1.05, 0.91, 0.90, 1.08, and 0.92

Mean fetal body weight (g): 5.54 A/5.65 M/5.43 F, 5.52 A/5.66 M/5.38 F, 5.30 A/5.43 M/5.16 F, 4.87 A/4.99 M/4.75 F, and 4.76 A/4.90 M/ 4.62 F

A = all fetuses; M = male; F = female

Single occurrences of external and visceral malformations were observed in DMF groups. However, there was neither a specific pattern of malformations nor a significant increase in the incidence of total malformations. There were no significant changes in the incidence of external or visceral variations. Statistically significant increases in the incidence of 2 skeletal variations, unossified or incompletely ossified supraoccipital and sternebrae, were seen in fetuses from the 200 and 300 mg/kg groups. A summary of fetal malformations and variations is provided in the table below. Incidences of findings are presented as number of fetuses (litters) affected [0, 50, 100, 200, and 300 mg/kg/day dose groups, respectively].

Number of fetuses (litters) examined

External exam: 244 (16), 290 (20), 281 (19), 277 (19), and 281 (20)

Visceral exam: 122 (16), 145 (20), 141 (19), 138 (19), and 141 (20)

Skeletal exam: 122 (16), 145 (20), 140 (19), 139 (19), and 140 (20)

Malformations

Exophtalmia bilateral: 0, 0, 0, 0, and 1 (1)

Encephalocele: 0, 0, 0, 0, and 1 (1)

Agnatia: 0, 0, 0, 0, and 1 (1)

Absence of nasal septum: 0, 0, 0, 0, and 1 (1)

Interventricular septum defect: 0, 1 (1), 0, 0, and 0

Diaphragmatic hernia: 0, 1 (1), 1 (1), 0, and 0

Hydronephrosis (bilateral): 0, 0, 0, 1 (1), and 1 (1)

Total number with malformations: 0, 2 (2), 1 (1), 1 (1), and 2 (2)

External variations

Hindlimb talipes: 0, 0, 0, 1 (1), and 0

Rudimentary tail: 0, 0, 1 (1), 0, and 0

Total number with external variations: 0, 0, 1 (1), 1 (1), and 0

Visceral variations

Dilated renal pelvis: 4 (2), 5 (5), 0, 1 (1), and 1 (1)

Dilated ureter: 17 (8), 6 (4), 5 (5), 4 (4), and 10 (4)

Total number with visceral variations: 17 (8), 10 (8), 5 (5), 5 (5), and 11 (5)

Skeletal variations

Skull

Parietals, incomplete ossification: 2 (1), 0, 0, 0, and 0

Supraoccipital, imcomplete ossification (moderate): 0, 1 (1), 8 (6), 52 (16), and 49 (17)

Supraoccipital, absent or imcomplete ossification (Severe): 0, 1 (1), 1 (1), 12 (9), and 70 (16)

Total number with skull variations: 2 (1), 2 (2), 9 (7), 64 (16), and 119 (20)

Sternebrae

5th absent or imcomplete ossification: 3 (2), 12 (6), 13 (7), 15 (11), and 32 (13)

2nd and 5th absent: 0, 1 (1), 0, 0, and 0

Ribs

13th short: 0, 0, 0, 0, and 1 (1)

Extra cervical: 2 (2), 2 (2), 1 (1), 1 (1), and 1 (1)

Extra lumbar: 11 (7), 8 (4), 7 (7), 4 (3), and 1 (1)

Vertebral centra, incomplete ossification: 8 (7), 11 (7), 26 (11), 19 (10), and 8 (4)

Total number with skeletal variations: 21 (11), 34 (13), 48 (16), 81 (19), and 125 (20)

DMF was not selectively toxic to the rat conceptus following oral administration. The NOAEL for maternal and developmental toxicity was 50 mg/kg/day. DMF was neither embryolethal nor teratogenic at doses up to 300 mg/kg/day.

Test substance : DMF, purity 99%
07.02.2006 (164)

Species : rabbit

Sex	:	female
Strain	:	other: Chbb: HM (Russian)
Route of admin.	:	gavage
Exposure period	:	Gestation days 6-18
Frequency of treatm.	:	Daily
Duration of test	:	
Doses	:	0, 46.4, 68.1, 200 µL/kg (ca. 44.1, 65, 190 mg/kg/day)
Control group	:	yes
NOAEL maternal tox.	:	65 mg/kg bw
NOAEL teratogen.	:	44.1 mg/kg bw
Method	:	other
Year	:	1976
GLP	:	no
Test substance	:	other TS

Method : No specific test guideline was reported; however, methods were according to FDA guidelines for reproduction studies for safety evaluation of drugs for human use.

The doses used for the study corresponded to ca. 1/45, 1/30, and 1/10 of the approximate 50% lethal dose. A total of 65 rabbits were used on the study with 24, 12, 18, and 11 rabbits used for the control, 44.1, 65, and 190 mg/kg/day groups, respectively. The rabbits were 20 to 47 weeks of age. The day of artificial insemination was designated as gestation day 0.

DMF was dissolved in aqua bidest and was administered at a dosage volume of 10 mL/kg. Clinical signs of toxicity, mortality, and food consumption were evaluated daily during the study. Body weights were recorded periodically throughout the study. The rabbits were sacrificed on gestation day 28 and were investigated by gross pathological examination. Uterine content was examined with respect to number of implantation sites, resorptions, number of live and dead fetuses, and the number of corpora lutea was counted. Sex, length, and weight of live fetuses and the respective placental weight was recorded. All fetuses were examined for external malformations, and for skeletal examination they were x-rayed in 2 levels (dorsoventral and lateral). The head of all fetuses were fixed in Bouin's solution and investigated according the to technique of Wilson.

Remark : Reliability: Medium because a suboptimal study design was used.

Result : All animals survived until scheduled termination.

Maternal toxicity was evident in the 190 mg/kg group. Reduced body weights, body weight gain, and food consumption occurred in this dose group. Three dams aborted, 1 on day 21, 1 on day 24, and 1 on day 28. At necropsy, the liver of 1 dam was a clay-like color.

At 190 mg/kg, fertility index, number of corpora lutea, number of implantations, and the ratio of live/dead fetuses were unaffected. Placental weights and fetal weights, as well as fetal length were significantly decreased. The incidence of malformed fetuses observed in 7 litters was increased (16/45 = 35.5%). Hydrocephalus internus (6 fetuses), exophthalmia (2 fetuses), ectopia visceralis (3 fetuses), hernia umbilicalis (7 fetuses), and cleft palate (1 fetus) were observed. Three fetuses showed multiple malformations.

At 65 mg/kg, a transient reduction in maternal food consumption was observed during the treatment period; however, this had no effect on body weight or body weight gain. Gross necropsy revealed a clay-like colored liver in 1 dam.

At 65 mg/kg, the mean number of implantations and percentage of live fetuses was decreased; however, a dose-response relationship was not evident. Fetal parameters, number and type of variations and retardations

were unchanged. Three malformed fetuses in 2 litters were found. This incidence was not statistically different from the control; however, the type of malformation (hydrocephalus internus) indicated a substance-related effect.

In the low-dose group (44.1 mg/kg), a transient reduction in maternal food consumption was observed during the treatment period without any effect on body weight or body weight gain. No substance-related pathological findings were recorded. Gestational and fetal parameters were unaffected. One malformed fetus (hydrocephalus internus) was found; however, this was in the range of the control.

The maternal NOEL was 65 mg/kg and the fetal NOEL was 44.1 mg/kg.

Test substance : DMF, purity not reported
03.02.2006 (9) (122)

Species : rat
Sex : female
Strain : Long-Evans
Route of admin. : inhalation
Exposure period : Gestation days 6-15
Frequency of treatm. : 6 hours/day
Duration of test :
Doses : 0, 18, 172 ppm
Control group : yes
NOAEL maternal tox. : 18 ppm
NOAEL teratogen. : 18 ppm
Method : other
Year : 1975
GLP : no
Test substance : other TS

Method : No specific test guideline was reported; however, methods of Lorke, D. (1963). Naunyn-Schmiedeberg's Arch. Exp. Pathol. Pharmakol., 246:147 and Lorke, D. (1965). Naunyn-Schmiedeberg's Arch. Exp. Pathol. Pharmakol., 250:360 were used.

Female rats weighed 200-250 g and were 2.5-3.5 months old at the beginning of the study. Two females were mated with one male overnight. Vaginal smears were prepared, and the day sperm were observed was designated as day 0 of gestation. At that time the females were housed singly.

There were 22-23 pregnant female rats per test group.

DMF was dissolved in polyethylene glycol 400. Control animals inhaled 20 mm3 polyethylene glycol/L air only. The DMF concentrations were determined by gas chromatography.

Cesarean sections were performed on gestation day 20. The average weight of fetuses/litter was determined. All fetuses were examined for external deformities. Approximately 1/3 of the fetuses were examined viscerally via the Wilson technique modified by Machemer and Stenger. The remaining fetuses were exenterated and abdominal and thoracal organs were evaluated. The fetuses were stained according to Dawson for skeletal examinations.

Remark : Reliability: Medium because a suboptimal study design was used.
Result : The analytically measured concentrations were 17.8 and 172.3 ppm DMF for the 18 and 172 ppm groups, respectively.

All animals survived until scheduled cesarean section. The pregnancy rate was 85, 100, and 100% for the control, 18, and 172 ppm groups,

respectively.

Fetal and maternal weights were reduced at 172 ppm. A summary of findings is presented in the table below [0, 18, and 172 ppm groups, respectively].

Maternal weight gain during gestation (g): 102.7, 105.7, and 103.6

Maternal weight gain during treatment period (g): 36.3, 36.9, and 33.5

No. of implantations: 12.0, 11.4, and 11.4

No. of fetuses: 9.5, 10.4, and 10.6

No. of resorptions: 2.5, 1.0, and 0.8

Avg. fetal weight (g): 4.07, 4.03, and 3.78

Fetuses with slight bone changes: 2.76, 2.95, and 2.30

Fetuses with deformities: 0.06, 0.10, and 0.10

Stunted fetuses (<3 g): 0.06, 0.20, and 0.20

The NOEL for maternal and developmental toxicity was 18 ppm.

Test substance : DMF, purity not reported
03.02.2006 (92)

Species	:	rat
Sex	:	female
Strain	:	other: Crl:CD
Route of admin.	:	inhalation
Exposure period	:	Gestation days 6-15
Frequency of treatm.	:	6 hours/day
Duration of test	:	
Doses	:	0, 30, 300 ppm
Control group	:	yes
NOAEL maternal tox.	:	30 ppm
NOAEL teratogen.	:	30 ppm
Method	:	other
Year	:	1992
GLP	:	no data
Test substance	:	other TS

Method : No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

Nulliparous female rats were 12-14 weeks of age upon arrival. Female rats were paired with male rats of the same strain 1:1, and mating was verified by the finding of sperm in the vaginal smear following overnight cohabitation. The day sperm was noted was designated as gestation day 0. Females were individually housed following mating.

There were 21 pregnant female rats per group.

Inhalation exposures were conducted in stainless steel and glass chambers (Rochester-type) with an effective volume of 760 L. DMF was generated by passing a stream of dry air through bubblers containing DMF. The air flow rate through the bubbler was monitored with a rotameter and was calibrated to volatilize the appropriate amount of test substance. The vapor-air mixture was diluted to the appropriate concentration with room air prior to entering the exposure chamber. Control rats were exposed to air

only. DMF concentrations were measured by IR analysis.

Maternal body weights were recorded on gestation days 0, 6-15, and 21. Clinical signs were recorded daily. Cesarean sections took place on gestation day 21. Corpora lutea were counted, fetuses were removed from the uterus, and the number and position of all live, dead, and resorbed fetuses were recorded. The fetuses were individually weighed, sexed, and examined for malformations. Approximately 2/3 of the fetuses from each litter were examined for visceral alterations. These fetuses were then eviscerated and stained for skeletal examinations. Approximately 1/3 of the fetuses were fixed in Bouin's solution and examined for neural and visceral defects using the technique of Wilson.

Comparisons between control and DMF-treated groups were made, where applicable, by the chi-square method. Body weights, body weight gains, numbers of corpora lutea, implantations of the dams, number of fetuses per sex, fetal and litter weights, crown-rump distances, and the number of ossification variations/fetus/litter were compared to control by the F-test and Student's t-test. When variances differed significantly, Student's t-test was appropriately modified using Cochran's approximation. Mean reproduction data were compared to control by the one-tailed t-test.

Remark : Reliability: Medium because a suboptimal study design was used.

Result : The mean exposure concentrations for this study were 0, 31.2, and 297 ppm for the 0, 30, and 300 ppm groups, respectively.

No mortality was observed during the study. No adverse clinical signs were noted in DMF-exposed rats. Maternal weight gains were reduced in the 300 ppm group. There were no test substance-related necropsy findings.

Reductions in the numbers of implantations, fetuses, and corpora lutea were observed in the 30 ppm group. Since these findings were not observed in the 300 ppm group, they were not considered to be DMF-related findings.

A summary of reproductive outcomes is provided in the table below [0, 30, and 300 ppm groups, respectively].

% Pregnant: 100, 100, and 100

Corpora lutea/female: 15.3, 14.6, and 14.4

Implantations/female: 14.2, 12.7, and 14.4

Resorptions/female: 0.5, 0.8, and 0.5

Live fetuses/female: 13.7, 12.0, and 13.9

Mean fetal weight (g): 5.5, 5.5, and 5.3

Viable fetuses/implantation site (%): 96.5, 94.5, and 96.5

Mean crown-to-rump (cm): 4.0, 4.0, and 4.0

Sex ratio: NR, NR, and NR

NR = Not reported

Soft tissue malformations indicated a high incidence with distended renal pelvis and/or ureters in all groups. The total incidence of fetuses and litters containing fetuses with soft tissue malformations were comparable between the control group and the DMF-treated groups. Skeletal

malformations were not unusual and included angulated, wavy, or extra ribs, fused thoracic vertebrae, split sternebrae, and misshapen scapula. These findings were distributed throughout the groups, were low in incidence, and do not suggest any relationship to DMF exposure. A summary of fetal malformations and variations is provided in the table below. Incidences of findings are presented as number of fetuses affected. The number in parenthesis represents percent of those examined [0, 30, and 300 ppm groups, respectively].

No. examined externally: 288, 251, and 291

Diaphragmatic hernia: 0 (0), 1 (0.4), and 0 (0)

Lens-vacuoles: 0 (0), 1 (0.4), and 2 (0.7)

No. examined viscerally: 97, 81, and 99

Distended renal pelvis: 31 (32.0), 17 (21.0), and 26 (26.3)

Distended renal pelvis and ureter: 6 (6.2), 4 (4.9), and 7 (7.1)

Distended renal pelvis, ureter, and bladder: 1 (1.0), 0 (0.0), and 0 (0.0)

Distended cerebral ventricles: 1 (1.0), 0 (0.0), and 0 (0.0)

No. examined skeletally: 191, 170, and 192

Misshapen scapula: 1 (0.5), 0 (0.0), and 0 (0.0)

Angulated ribs: 1 (0.5), 2 (1.2), and 0 (0.0)

Wavy ribs: 0 (0.0), 1 (0.6), and 0 (0.0)

Cervical rib: 0 (0.0), 1 (0.6), and 0 (0.0)

Fused thoracic vertebrae: 0 (0.0), 1 (0.6), 0 (0.0)

Split sternebrae: 0 (0.0), 0 (0.0), 1 (0.5)

Maternal and fetal toxicity were evident at 300 ppm as evidenced by reduced maternal and fetal weight. The NOEL for both maternal and fetal toxicity was 30 ppm.

Test substance : DMF, purity not reported

14.02.2006 (105)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

Type	:	
In vitro/in vivo	:	In vivo
Species	:	other: Swiss mice and Wistar AG rats
Sex	:	
Strain	:	
Route of admin.	:	i.p.
Exposure period	:	7 weeks
Frequency of treatm.	:	5 injections per week for a total of 36 injections
Duration of test	:	
Doses	:	50 mg/kg
Control group	:	yes
Method	:	other
Year	:	1971

GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	No specific test guideline was reported. An acute lethality study was conducted as well as a repeated dose study. MMF was dissolved in isotonic saline solution (50 g/100 mL). In the acute study, mortality was recorded. Clinical signs were recorded periodically. Animals which died as a consequence of administration, as well as the survivors which were sacrificed 30 days after administration, were subjected to necropsy. Ten organs, including the testicles, were removed for pathoanatomic examination. In the repeated dose study, 20 male rats received 50 mg/kg (1/20 of the sublethal dose). An additional group of 20 male rats received isotonic saline, and another 20 male rats received no treatment. Rats received 5 injections per week for approximately 7 weeks. The rats received a total of 36 injections. Clinical signs and body weight were recorded. After 15 injections, 5 rats/group and after the 36th injection, all remaining rats were placed in metabolism cages. Urine was collected after 24 hours in the cages. After the 24-hour period, the rats were sacrificed. Blood was taken for hematological evaluation. Pathoanatomic evaluation was conducted on 10 organs, including the testicles.
Remark	:	Reliability: Not assignable because limited study information was available.
Result	:	In the acute study, an adverse effect on the seminiferous tubules was observed in rats and mice treated with MMF. The germinal epithelium was detached from the vitreous hyaline and numerous spermatogonia and spermatocytes were necrosed. These testicular lesions were not observed in the survivors sacrificed 30 days after the administration of MMF. In the repeated dose study, pathoanatomic examination revealed no microscopically demonstrable lesions. The authors state that their results confirm the findings of Lechat, P. et al. (1960). C. R. Acad. Sci. Paris, 251:1937-1939 and Wallon, D. et al. (1960). Le Sang, 31(9):871-879. In these studies, the authors reported that the testicular lesions were the result of karyoclastic activity which was also manifested in the bone marrow, ileum, and liver.
Test substance	:	N-methylformamide, purity not reported

07.02.2006 (24)

Remark	:	Data from this additional source were not summarized because insufficient study information available.

03.02.2006 (131)

Remark	:	Supporting data on dimethylformamide (DMF).

03.02.2006

Type	:	
In vitro/in vivo	:	In vivo
Species	:	rat
Sex	:	male/female
Strain	:	other: CD
Route of admin.	:	dermal
Exposure period	:	Up to 164 days
Frequency of treatm.	:	Daily
Duration of test	:	
Doses	:	0, 500, 1000, 2000 mg/kg/day
Control group	:	yes
Method	:	other

Year	:	1973
GLP	:	no
Test substance	:	other TS

Method : No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

There were 10 male and 20 female rats per group.

Three treatment sequences were employed during the experiment as follows:
Sequence 1: male and female rats were treated with 500, 1000, or 2000 mg/kg DMF during test day 0 through 28 (pre-mating period), and with distilled water for the rest of the study.
Sequence 2: female rats were treated with 500, 1000, or 2000 mg/kg DMF during both mating and gestation periods, and with distilled water during pre-mating and lactation periods. Males received distilled water throughout the entire investigation.
Sequence 3: male and female rats were treated with 500, 1000, or 2000 mg/kg throughout the entire investigation (premating, mating, gestation, and lactation periods).
Two control groups (distilled water) were also used in the study.

Premating occurred during test days 0-28 and again during test days 29-56. The first mating occurred during test days 57-72, the first gestation was during test days 73-94, the first lactation was during test days 95-116. A rest period occurred during test days 117-127. Mating occurred again during test days 128-143 followed by the second gestation during test days 144-164.

DMF was applied during the dosage periods to the skin on the backs of the animals. The skin had been previously clipped of hair.

Body weights, clinical signs, and mortality data were collected periodically during the study. Parental animals were also evaluated for fertility, length of gestation, and lactation performance.

Mating trials were initiated when the rats were 100 days old (56 days on test). Females were caged in pairs and mated with a male from within the same treatment group.

The first litters obtained (F1a) were weaned 21 days post-partum. Pups were examined for physical abnormalities at birth and again at weaning. Records of live birth and pup survival at various stages of the lactation period were maintained.

Parental animals were then given a 10-day rest period and then mated again. All females were sacrificed on the 20th day of the second (F1b) gestation period. Uterine contents were examined. Fetal swellings and implantation sites were counted, with special attention being paid to resorption sites or uterine abnormalities. The number of corpora lutea were also counted. Each fetus was given an external examination. All fetuses from the control and 2000 mg/kg DMF groups were examined for either skeletal or internal development. Where possible, equal number of fetuses of each sex from each litter were examined by each method. Evaluation of skeletal development was conducted by the Hurley's method. Internal development was evaluated using the free-hand razor blade section technique of Wilson and Warkany.

Males and females which failed to become pregnant were sacrificed at the conclusion of the second breeding period. Females that conceived the F1b litter were sacrificed on the 20th day of gestation period. A complete gross

pathological examination was performed on all sacrificed animals and samples of liver, kidney, and gonadal tissues from 10 male and 10 females of each group were preserved. Microscopic examination of these tissues was conducted for 10 males and 10 females of the control and high-dose group or each sequence.

Remark : Reliability: High because a scientifically defensible or guideline method was used.

Result : Body weight gains were reduced in the 1000 and 2000 mg/kg males during the pre-mating treatment periods (sequences 1 and 3). The final body weights of males receiving 1000 or 2000 mg/kg (sequence 3) were less than the control males. Females treated with 2000 mg/kg during sequence 3 gained less weight than control females during the pre-mating phase. The final body weights of 2000 mg/kg females were less than the control females during both sequences 2 and 3.

An increase in mortality occurred in the 1000 and 2000 mg/kg groups during dosage sequence 3. Lung consolidation was noted during the gross pathology exam and death was attributed to chronic respiratory infection.

There were no untoward behavioral observations noted among parental animals during the investigation. Gross and histopathologic examination of the control and high-dose animals revealed no differences between control and test groups.

No differences between the control and test groups were noted in the parameters assessed for reproductive ability.

Fewer pups were delivered and retained during the lactation period by females given 2000 mg/kg during sequences 2 and 3. Pup survival indices, calculated at various intervals during lactation, revealed a reduction of survival for pups delivered by dams given 1000 or 2000 mg/kg during sequences 2 and 3. Weanling body weights for the test groups were similar to the controls.

For the F1b litters, administration of 2000 mg/kg in sequences 1, 2, and 3 resulted in a reduction in the number of viable pups per litter. These decreases appeared to be the result of reduced numbers of corpora lutea and implantation sites per female in those groups. Treatment with 500 or 1000 mg/kg did not affect ovulation, implantation, or fetal survival. Fetal body weights were not affected by prenatal exposure to DMF. No differences between control and treated groups were noted in the fetal external examination. An increase in the percent of fetuses with incompletely or non-ossified sternum sections was observed among pups from the 2000 mg/kg groups. These values were within the expected range for the testing laboratory and were therefore not attributed to the administration of DMF.

Test substance : DMF, purity not reported

14.02.2006 (76)

Type :
In vitro/in vivo : In vivo
Species : mouse
Sex : male/female
Strain : other: CD-1®(ICR)BR
Route of admin. : drinking water
Exposure period :
Frequency of treatm. : Continuously in the drinking water
Duration of test :
Doses : 0, 1000, 4000, 7000 ppm (ca. 219, 820, and 1455 mg/kg/day)
Control group : yes
Method : other
Year : 1998

GLP : yes
Test substance : other TS

Method : No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

The RACB protocol (Reproductive Assessment by Continuous Breeding protocol) was used and consisted of 4 segments: 1) a dose range-finding phase, 2) an F0 cohabitation and lactation phase, 3) a crossover mating trial of the F0 generation (conducted if F0 reproductive performance was affected), and 4) a fertility assessment of the F1 generation (born and reared during the F0 lactation phase).

Mice were 6 weeks old upon arrival.

Range-finding study:
Dose levels used in the range-finding study were 0, 2500, 5000, 7500, 10,000, and 15,000 ppm in deionized, filtered drinking water. Male and females (8 per sex per group), 8 weeks of age, were used in the range-finding study. Food and water consumption and body weights were measured weekly during the 2-week exposure period. At the end of the 2 weeks, the animals were sacrificed with no further data collection.

F0 cohabitation and lactation study:
Male and female mice, 11 weeks of age, were assigned to 1 of 4 dose groups. The control groups consisted of 40 males and 40 females, and the test groups consisted of 20 males and 20 females. Doses used for this study were 0, 1000, 4000, and 7000 ppm. Body weights and food and water consumption were monitored periodically throughout the study. During week 1 of this study, the animals were individually housed. During weeks 2-15, mice were housed in breeding pairs within dose groups. Newborn litters were sacrificed immediately after evaluation. Data collected included litter interval, number, sex, weight of pups per litter, number of litters per breeding pair, and the postnatal day (PND) 0 body weight. The breeding pairs were separated at week 16 and F0 females were allowed to deliver and rear the final litter until PND 21. Pups were sexed, counted, and weighed periodically through the postnatal phase. On PND 21, randomly selected F1 pups from each dose group were weaned and housed in same-sex pairs by dose and saved for the F1 fertility assessment phase (described below). After completion of the cohabitation and lactation phase, all F0 animals were maintained on their respective treatment until scheduled sacrifice after the completion of the crossover mating phase.

Crossover mating trial (conducted because fertility was affected in the F0 cohabitation and lactation study):
This study was conducted using the control and high-dose mice. Three breeding groups of F0 animals were created: 1) control male x control female, 2) high-dose male x control female, and 3) control male x high-dose female. Beginning at week 23 of treatment, mice were cohabited until a vaginal copulatory plug was observed or for 1 week. No treatments were administered during cohabitation. Mice were singly housed at week 24 and dosing resumed. Upon delivery of each litter, lethality, gestation length, sex, number, weight of pups, and dam weight were recorded. All newborn litters were sacrificed following evaluation. After all litters had been delivered, vaginal smears were recorded for 12 days. At week 29, all F0 male and female mice were weighed, sacrificed, and necropsied. Liver and paired kidney weights were recorded for both sexes. Right testis, right epididymis, prostate, and seminal vesicles with coagulating glands were also weighed. The right ovary with attached oviducts was weighed in females. All tissues were fixed and embedded in paraffin. Sperm evaluations from the right testis included manual assessments of motility,

concentration, and morphology. Homogenization-resistant spermatid head were counted from the left testis. Histopathologic evaluations were conducted on all livers, right and left kidneys and adrenals, the right testis and epididymis, prostate, seminal vesicles, ovary, and any gross lesions noted during necropsy.

F1 fertility assessment:
At weaning (PND 21), randomly selected F1 pups from the control, 1000, 4000, and 7000 ppm groups were housed 2 per cage by sex within dose group. On PND 22, the pups began directly receiving DMF via the drinking water. At 74±10 days of age, males and females in the control or treated groups were cohabited as nonsibling breeding pairs until a vaginal copulatory plug was observed or for 1 week. Although 20 non-sibling pairs was the goal, reduced survival in the high-dose group, allowed only for 15 pairs, some of which were siblings. Litter data were collected as described above for the F0 adults in the crossover mating study. After delivery of the F2 litters, vaginal smears were collected for 12 days for the F1 females. Body weight and food and water consumption were recorded periodically. At necropsy, F1 males and females were weighed and data collected as described above for the F0 animals. Histopathological evaluations were conducted on all livers, right and left kidneys and adrenals, the right testis and epididymis, ovary, and any gross lesions noted during necropsy.

Selected F2 litters were preserved on PND 1 and evaluated for whole body skeletal malformations and soft tissue malformations of the head. Selected adult F1 males and females were evaluated for skeletal malformations.

Statistical analysis:
Most hypotheses were tested using the Williams' modification on Dunn's or Shirley's nonparametric multiple comparison procedures. Jonckheere's test was used to ascertain whether there was sufficient evidence of a dose-related response to apply Shirley's test. For data expressed as a proportion, the Cochran-Armitage test was used to test for a dose-related trend, and pairwise comparisons were performed using a Chi-square test.

Remark : Reliability: High because a scientifically defensible or guideline method was used.

Result : Range-finding study:
Treatment-related deaths occurred at doses of 10,000 (3 males) and 15,000 ppm (7 males and 3 females). Body weight was decreased in the remaining 15,000 ppm mice. Water consumption was decreased in both sexes at weeks 1 and 2. Doses of 1000, 4000, and 7000 ppm were chosen for the continuous breeding study.

Cohabitation and lactation studies (continuous breeding phase):
For F0 animals, there was no increased incidence of mortality and no dose-related clinical signs of toxicity. There was no effect on male body weight, food consumption or water consumption. Female body weight was significantly reduced at 7000 ppm at weeks 8 and 16, reflecting at least in part the non-pregnant status in 20-40% of the animals. For those mice that delivered a litter, body weight was affected by treatment at all doses by week 16.

During the lactational period, relative maternal feed consumption was significantly depressed at 7000 ppm on PND 0-4, at 4000 ppm or more midlactation, and at 1000 ppm or more on PND14-21. Relative maternal water consumption exhibited a similar, but more pronounced, effect.

At 7000 ppm, fertility was reduced in the first litter to 90%, compared to 100% in the controls. Over time, this treatment-related effect increased. By the final litter, fertility was reduced to 55% at 7000 ppm and reduced fertility was also noted at 4000 ppm. The average number of litters per pair, average litter size, proportion of pups born alive, and average pup

weight were all reduced compared to control pairs. There was no effect on these parameters in the 1000 ppm group. A summary of reproductive outcomes is provided in the table below [0, 1000, 4000, and 7000 ppm groups, respectively].

Number of breeding pairs: 38, 20, 20, and 20

Percent fertile (1st litter): 100, 100, 100, and 90*

Percent fertile (final litter): 92, 95, 70*, and 55*

Cumulative days to litter (1st litter): 21.7, 24.5, 28.1, and 23.1

Cumulative days to litter (final litter): 103, 105, 104, and 104

Litters per pair: 4.9, 4.8, 4.5*, and 3.8*

Live pups per litter: 11.8, 11.8, 7.5*, and 5.3*

Percent of live pups: 98, 99, 76*, and 71*

Live pup weight (g): 1.58, 1.55, 1.30*, and 1.27*

Adjusted live pup weight: 1.59, 1.55, 1.30*, and 1.26*

* = $P<0.05$, pairwise comparison to control

Pups born to DMF-treated pairs had external malformations and other abnormalities, including domed head and hematomas along the nose and on the head. Those pups most severely affected died shortly after birth, and many were cannibalized prior to examination. The proportion of litters with one or more pups with an abnormal appearance was 7.9, 10.5, 90.0, and 77.8% for the 0, 1000, 4000, and 7000 ppm groups, respectively. The reduction in the proportions of litters with malformed pups in the high-dose group, compared to the mid-dose group, was influenced by the decreased fertility, increased prenatal death, and postnatal cannibalism observed in the high dose group.

During the lactation phase, average post-natal survival was reduced in the 4000 and 7000 ppm groups. Live pup weight, reduced at birth, was affected only infrequently during the preweaning period.

Crossover mating trial and F0 necropsy:
No differences were detected in DMF-treated groups of either sex for comparisons with controls. The control group had a lower than usual pregnancy rate which resulted in fewer control litters, thus affecting the power of statistical analyses and the strength of the conclusions. Although no differences were observed between the treated groups and controls, differences between the treated groups were noted. Females treated with 7000 ppm (and mated with control males) produced fewer live pups per litter (5.5±1.0 vs. 10.2±1.2) when compared to the males treated with 7000 ppm (and mated with control females). Pup weights were also lower in the pups of dosed females compared to those sired by treated males. These data suggest that the female was the sex affected by DMF exposure. A summary of reproductive outcomes (means/litter unless otherwise noted) is provided in the table below [Control male x control female, 7000 ppm male x control female, and Control male x 7000 ppm female, respectively].

Percent fertility: 50 (8/16)a, 69 (11/16), and 55 (11/20)

Live pups per litter: 8.1 (8)b, 10.2 (11), and 5.5 (11)*

Live pup weight (g): 1.56 (6)c, 1.63 (11), and 1.44 (10)

Proportion of pups born alive: 0.73, 0.94, and 0.68

Adjusted live pup weight (g): 1.61d, 1.66, and 1.38**

Average dam weight (g): 40.30, 41.42, and 40.74

Average days to litter: 21.6, 22.0, and 21.6

a = number of deliveries/number cohabited
b = numbers in parentheses are the number of dams delivering litters
c = numbers in parentheses are the number of litters with live pups
d = body weight was adjusted statistically to account for differences in litter size
* = treated groups differ at P<0.075l ANOVA is P<0.07
** = treated groups differ from each other at P<0.05; differs from control at P=0.09.

Pups born to the treated females exhibited the same spectrum of malformations as observed during the continuous breeding phase (see the table below). Incomplete ossification of the cranial bones accounted for 82% of the malformations observed in the control male x control female group, and 97% of the malformations in the 7000 ppm male x control female group, but was not observed in the control male x 7000 ppm female group. Malformations observed in the control male x 7000 ppm female group included abnormal ossification of the cranial plates, abnormal suture formation in the cranium, and abnormal or incomplete formation of the sternebrae. Examination of 95 heads from randomly selected pups revealed that 23.1% of the pups born to DMF-treated mothers had malformations, including agenesis of the cerebrum, agnathia, abnormally shaped centrum or cranium, cleft palate, or enlarged cerebral ventricles. Head malformations from the other 2 groups were accounted for solely by the finding of enlarged nasal passages.

[Control male x control female, 7000 ppm male x control female, and Control male x 7000 ppm female, respectively]

Proportion of litters with 1 or more externally malformed pups: 12.5%, 0.0%, and 90.9%

Proportion of litters with 1 or more internally malformed pups: 83.3%, 81.8%, and 100%

Percent of pups (within litters) with skeletal malformations: 40%, 38%, and 95%

There was no effect on the length of the estrous cycle or stage frequency distribution; however, 86% of the controls had 4- or 5-day estrous cycles, compared to 66% after DMF exposure. Thus, the number of animals having normal cycles was affected by DMF.

At necropsy, DMF-treated F0 females had significantly depressed body weights. Male body weights were not affected. Male liver weights were increased at all doses. Female absolute and relative liver weight and kidney plus adrenal weights were increased at all doses. Histopathologic examination of animals with gross liver lesions (2 mid-dose females and 2 high-dose males) revealed centrilobular hepatocellular hypertrophy, which was considered treatment-related. Caudal epididymidal weight was significantly increased at all doses of DMF. A slight decrease in testicular spermatid concentration in the DMF-treated animals was significant in the low and high doses, with a significant trend present. DMF had no adverse

effect on epididymal spermatozoan concentration, motility, or morphology. Histopathologic examination of the reproductive organs found no findings related to DMF treatment. Therefore, the authors conclude that the effect on testicular spermatids was likely a Type II error and not biologically relevant.

Growth and survival of F1 juveniles:
The proportion of F1 pups born alive in the final litter and postnatal survival on postnatal day (PND) 4 were reduced in the mid- and high-dose groups and continued to decline throughout the lactation period. Pup weight during lactation was reduced in the mid- and high-dose groups prior to PND7 and may have contributed to decreased survival rate. The F1 pups in the mid- and high-dose groups also exhibited craniofacial malformations. Pups that were severely malformed did not survive the preweaning period. The surviving F1 pups were closely examined and those in the mid- and high-dose groups were small and appeared to have foreshortened, domed heads.

After weaning, pups were randomly selected for rearing and inclusion in the reproductive performance evaluation of the F1 generation. Both male and female body weights were reduced in the mid- and high-dose groups throughout the remainder of the study. Food consumption was unaffected in the F1 generation. Water consumption was increased for the males in the mid- and high-dose groups. Estimated mean exposure to DMF was 259, 1023, and 1934 mg/kg/day for the 1000, 4000, and 7000 ppm groups, respectively, with females receiving slightly more DMF than males.

Reproductive performance of the second generation:
The mating index was significantly decreased at 7000 ppm. Fertility was reduced in the 4000 and 7000 ppm groups. The average days to litter was increased, and the number of live pups per litter, pup body weight, and the proportion of pups born alive was decreased in the 4000 and 7000 ppm groups. Live pup weight was also decreased in the 1000 ppm pups. A summary of reproductive outcomes (means/litter unless otherwise noted) is provided in the table below [0, 1000, 4000, and 7000 ppm groups, respectively].

Percent fertile: 90 (18/20)*, 90 (18/20), 56 (10/18)*, and 53 (8/15)*

Live F2 pups per litter: 11.3, 11.8, 4.9*, and 4.1*

Proportion of pups born alive: 1.00, 0.99, 0.74*, and 0.56*

Live F2 pup weight (g): 1.59, 1.48*, 1.30*, and 1.32*

Adjusted live F2 pup weight (g): 1.61, 1.52*, 1.21*, and 1.23*

Average dam weight (g): 34.9, 34.7, 30.2*, and 28.9*

Average days to litter: 21.2, 21.6, 23.0*, and 23.5*

* = statistical significance for comparisons of dosed groups to controls ($P<0.05$).

F2 pups born to DMF-treated F1 pairs exhibited malformations similar to those observed for the F1 litters of F0 pairs. The proportion of litters with 1 or more externally malformed pups was 0, 27.7, 60, and 75% for the 0, 1000, 4000, and 7000 ppm groups, respectively.

F1 estrous cycles were monitored after the birth of the F2 pups. Females in the 7000 ppm group had significantly longer cycles and tended to be in either metestrus or diestrus longer than control animals.

At necropsy, F1 male and female body weights were reduced in the 4000 and 7000 ppm groups. Absolute and relative liver weights were significantly increased in all DMF-treated animals of both sexes. Female relative kidney plus adrenal weight was increased in the 4000 and 7000 ppm groups. Histopathological examination of the animals (in low and high dose groups) with gross lesions revealed treatment-related centrilobular hepatocellular hypertrophy. These finding indicated a general toxicity at 1000 ppm or more.

Evaluation of F1 reproductive tissues revealed some significant effects in males but not females. Relative prostate weight was decreased at all doses, as was absolute prostate weight in males in the 4000 and 7000 ppm doses. Epididymidal spermatozoa concentrations was decreased at 7000 ppm, but no other significant effects of treatment were noted for andrologic parameters. Relative ovary weight was increased in the 4000 ppm females due to the presence of cystic ovaries in 2 animals, but was not considered treatment-related.

Developmental effects observed at delivery were confirmed in surviving F1 animals. Malformations observed in the mid- and high-dose animals consisted of abnormal or incomplete ossification of the cranial plates, abnormal cranial suture formation, and abnormally formed sternebrae. Histopathologic evaluation of additional F1 animals in the mid- and high-dose group revealed dysplasia of the cranial bones, primarily at the midline.

Significant reproductive and developmental toxicity was observed in both generations at 4000 and 7000 ppm DMF in the presence of some general toxicity. The liver appeared to be the primary non-reproductive target organ. Reduced F2 pup weight was noted at 1000 ppm DMF.

The NOEL for the parental generation was < 1000 ppm. The NOEL for the F1 offspring was 1000 ppm and the NOEL for the F2 offspring was < 1000 ppm.

Test substance : DMF, purity > 99%

07.02.2006 (57)

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

6.1 ANALYTICAL METHODS

6.2 DETECTION AND IDENTIFICATION

7.1 FUNCTION

7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED

7.3 ORGANISMS TO BE PROTECTED

7.4 USER

7.5 RESISTANCE

8.1 METHODS HANDLING AND STORING

8.2 FIRE GUIDANCE

8.3 EMERGENCY MEASURES

8.4 POSSIB. OF RENDERING SUBST. HARMLESS

8.5 WASTE MANAGEMENT

8.6 SIDE-EFFECTS DETECTION

8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER

8.8 REACTIVITY TOWARDS CONTAINER MATERIAL

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10.1 END POINT SUMMARY

10.2 HAZARD SUMMARY

10.3 RISK ASSESSMENT